

Infantile Genetic Agranulocytosis

(Agranulocytosis infantilis hereditaria)

A New Recessive Lethal Disease in Man

By

ROLF KOSTMANN

Uppsala

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INFANTILE GENETIC AGRANULOCYTOSIS

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INAUGURAL DISSERTATION

BY

ROLF KOSTMANN

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By due permission of the most experienced Medical
Faculty of the University of Uppsala, to be publicly
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ALMQVIST & WIKSELLS BOKTRYCKERI AB

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To the Memory of my Father

EDVIN SVEN VILHELM KOSTMANN

1885-1948

Preface

This investigation was started in 1949 when I was director of the Division of Pediatrics at the Army Hospital in Boden. The officials of the county of Norrbotten and, especially, the administrative director of public health, Mr. K. G. Wiklund, have taken an active interest in my work and contributed a substantial grant. The cost of this project was also in part defrayed by the Swedish Medical Research Council and the foundation of Ernst and Hilma Fredga.

As any other investigation, this one could not have been completed without the cooperation of a number of different institutions and individual scientists. Among the latter, I want to mention especially H. Magnusson, M.D., late Director of the Sachs' Hospital for Children in Stockholm and Professor B. Vahlquist, M.D. Uppsala. The cooperation in the genetic analysis of the material by Jan A. Böök, M.D., Ph.D., Uppsala, has been invaluable.

N. G. Nordenson, M.D., Associate Professor of Hematology, and F. Wahlgren, M.D., Associate Professor of Pathology, both at the Southern Hospital in Stockholm, have greatly contributed by the analyses of the blood, bone marrow and histological specimens.

The help and encouragement received from all of them is gratefully acknowledged.

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Introduction

In this work, a new disease, *infantile genetic agranulocytosis*, will be described. As a rule, the outcome of this disease is fatal within the first year of life. The children succumb to infections of various kinds, most commonly manifested as septic skin conditions. The affected children have been discovered in a certain district in Norrbotten, the northernmost province of Sweden (see Fig. 1). The disease appears to depend primarily on a single recessive gene difference.

Chapter I

A. Collection of Data

In March 1949, a *two months old girl* (case A9) was admitted to the Children's Hospital in Boden, one of Sweden's northernmost towns. *She displayed multiple abscesses in the skin, otitis, mastoiditis, and high fever. She had complete agranulocytosis.* With antibiotic therapy, the infection could be counteracted, and the child was kept alive for almost four months. During the whole course of her disease, almost complete agranulocytosis was present. *The mother had earlier given birth to eight children; of these, four had died as infants, 26, 29, 30 and 32 days old, respectively. In the last few weeks of life, three of these four children had high fever, as well as furuncles and abscesses similar to those of the ninth child now in the hospital.* Two of these children died in the infirmary of Överkalix with the diagnosis of carbuncle and pemphigus, respectively. The third child died at home, where it was under the observation of the district nurse. The fourth child also died at home, with the diagnosis of pneumonia. *The mother claimed that similar diseases had occurred among infants in her husband's family, and that all affected children had died.*

As it seemed probable already from the start of my inquiry that this disease was genetically determined, that it appeared in children, predominantly infants, and that it usually led to death, a preliminary investigation of the family of the father was undertaken. All infants who had died by the age of six months were registered as suspects of the disease.

In the last generation, 83 children had been born, divided between 18 families. Of these 83 children, 14 died before the age of six months. No further children had died by the age of one year. This corresponds to an infant mortality of 168.7 per thousand. All children of the last generation were born after 1930 within the area of the Överkalix church registry, and all except two had died within this area. The infant mortality in this area within the last 18 years had been 225 dead of 4035 born, i.e. 55.8 per thousand. *Thus, the infant mortality in the generation of the family concerned had been three times as high as in the municipality as a whole.*

None of the 14 children had died as newborn. If one does not include those that died during the first week of life, the following figures are obtained:

For the municipality:	152 dead in 4035 born = 37.7 per thousand
In the family:	14 dead in 83 born = 168.7 per thousand.



Fig. 1.

The most obvious symptoms of the first case were furuncles, abscesses and skin infiltrations, with or without sores and fusions. If such signs had appeared in earlier, not-diagnosed cases, they ought to have been observed by the parents and others who had seen the children during the disease.

By questioning parents, district nurses, and doctors concerning the histories of the dead children, it could be established that eight of them had displayed symptoms similar to those of the first case. All of them had had furuncles, abscesses, phlegmons and high fever.

One of the cases discovered in this manner (case D1), had been treated in a hospital (Gällivare) and died there. According to the hospital record, the cause of death was agranulocytosis. Blood slides and pathologic-anatomic preparations had been kept, and an examination of these revealed changes identical to those of the first observed case.

A continued, systematic survey was made in the church records of the claimed causes of death of all infants that had died in the municipality during the last eighteen years. In cases where suspicious causes of death were present, more detailed information concerning the disease and manner of death was obtained from parents, physicians and nurses. For the same purpose, inquiries concerning individuals with suspicious diseases and causes of death were made in all neighboring hospitals. During the course of this investigation, a few additional individuals with this disease were discovered. The one that was most valuable for the investigation (case R4) had been referred to the hospital in Boden by a physician—Dr. *Bjarke*, to whom I am greatly indebted—who had recognized the disease through my first publication (Kostmann 1950).

All now-living families affected by the investigation have been visited by me, and I have personally recorded all information. The catamnestic statements were given by parents of the deceased children, and complementary information was provided by physicians, district nurses and midwives.

The genealogic data have been collected through the parish church registers. These investigations were partly performed by me, and partly by my assistant. Investigations were also made in the province archives in Härnösand, where the old registers are kept. During this work, a total of some 700 individuals has been investigated.

All parents and siblings of those children who had been judged to suffer from the disease or had died from it have been examined. In addition to a general physical examination, a blood examination was included, consisting of a hemoglobin determination, a white blood cell count, and a differential count. In one family (family A), an examination of sternal punctates of both parents has been undertaken. In two families (families

D and F), the same examination has been performed on the mothers. All these individuals displayed normal findings and were also generally healthy.

It should be added that all unaffected members of the families, adults as well as children, were physically and mentally well-developed individuals. All the families lived under fairly good economic conditions and had good, clean homes. Most of them could, without difficulty, be classified as belonging to the ordinary Nordic type. Only a few ancestors of Finnish or Lappish derivation were disclosed.

B. Classification of the Affected Individuals

With regard to the completeness of the examination and the degree of diagnostic certainty, the affected children have been classified into two groups:

- Group I. *Six children* who have been observed in hospitals and who have been subjected to more detailed pathologic studies.
- Group II. *Eight children* who, on account of catamnestic and genealogical information, have been judged as affected with the same disease entity. All have died as infants in septic diseases and have had furuncles, abscesses, and phlegmons.

The relationship of the different individuals are shown in the pedigrees (Fig. 15, p. 67). The sibships have been noted by capital letters and individuals by Arabic figures according to birth rank.

Chapter II

The Affected Children of Group I

CASE A9

Girl. Born January 7, 1949. Died June 26, 1949. Normal delivery. Weight at birth, 3000 g. Vaccinated against tuberculosis. She was breast-fed for $1\frac{1}{2}$ months. At the age of three weeks, she fell ill with hoarseness and fever; was treated with steam and sulfa drugs, 0.125 g. $\times$ 5 for three days, and recovered. At the age of four weeks, she had a right-sided otitis. She was taken to the local infirmary, and treated with penicillin. At the age of $1\frac{1}{2}$ months, she was transferred to the otologic department of the hospital in Boden. She had mastoiditis on the right side and was highly febrile. The mastoid cell system was large for her age, and the posterior wall of the auditory canal was almost completely destroyed. The cell system was entirely removed, and the wound cavity was filled with penicillin. The wound was sewn primarily, and a penicillin tamponade was placed in the auditory canal. She became free of fever and the recovery appeared normal. Three weeks after the operation, she got high fever, multiple boils (furuncles) and a complete agranulocytosis. She was transferred to the children's hospital, now 2 months and 10 days old.

Her general condition was strongly affected. She was highly febrile and pale-cyanotic. There were eight elevated, strongly reddish, furuncle-like eruptions the size of hazelnuts on the lower part of the abdomen and on the thighs. Nearly all had centrally a bluish-grey miscoloring about the size of a pea with blister formation or a pasty, necrotic sore of the same size (Fig. 2, p. 12). The superficial lymph nodes, liver and spleen could not be felt. The blood examinations have been summarized in Fig. 3 and Table I. Cultures from the blisters yielded growths of a pathogenic strain of *Staphylococcus aureus*, streptomycin sensitive but penicillin and sulfa resistant. A blood culture was negative. Wassermann reaction in blood negative. Mantoux 1 mg negative. She belonged to blood group A₁MNS Rh₁ (father: A₁MP Rh₁, mother: OMN Rh₁). Skeletal X-ray was negative. She was treated with streptomycin and blood transfusions. After ten days, her temperature returned to normal. Her general condition had improved considerably, and the furuncle-like skin changes had assumed a brown or blue-grey color. The central parts had been sloughed off, and the sores had cleaned up and were partly healed.

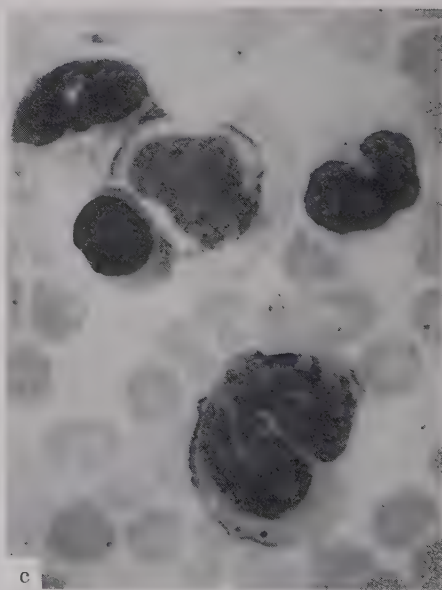
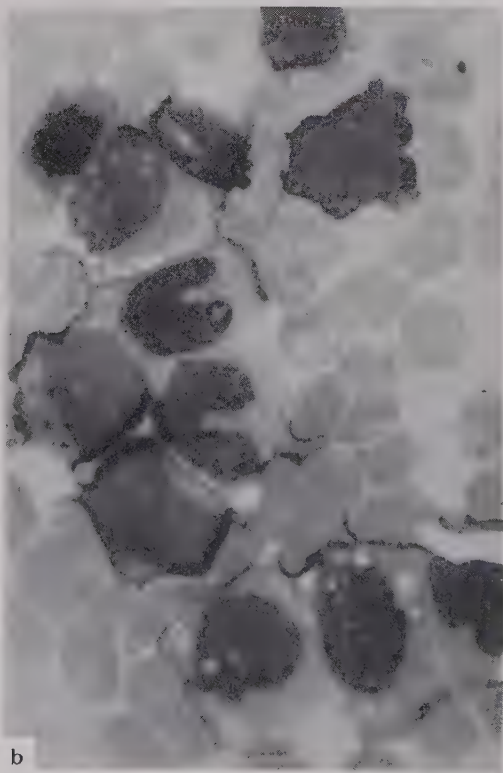


Fig. 2. Case A9. *a*, *b* and *c*, microphotographs of bone marrow smear. Vacuolisation, atypical nuclei and predominance of myelocytic cells.

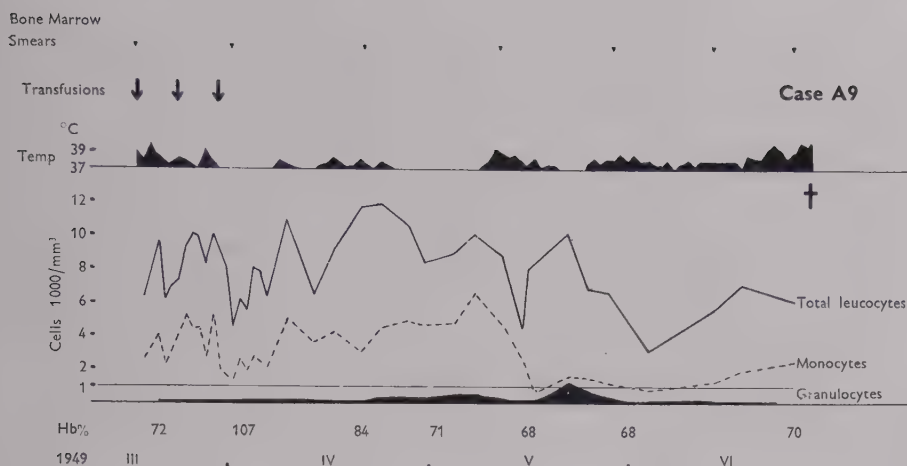


Fig. 3. Case A9. Graphical representation of the blood values during the course of illness.

During the next few weeks, her general condition was relatively unaffected despite sub-febrility and signs of infection of the upper respiratory tract. However, the skin color remained pale and tonus and turgor were reduced.

At the age of four months, new furuncles appeared on the abdomen, back, thighs and gluteal regions. The temperature rose again to over 39°C. Her general condition became worse. Enlargement of the liver, spleen and lymph nodes could not be observed at this time, either. After renewed streptomycin treatment for ten days, the temperature dropped again. The local skin processes improved.

The operation wound behind the right ear had healed. The cartilaginous part of the ear channel had sequestered and suppuration continued. An easily-bleeding polypus had formed in the ear channel.

At the age of 4½ months, the child was transferred to the Sach's Hospital for Children in Stockholm.

During the continued course of the illness, the fever was constantly about 38°C. Occasionally, new furuncles appeared. Incisions of melted, ripened furuncles and abscesses were made. Moderate quantities of hemorrhagic pus were liberated. In cultures from this pus, there still grew pure strains of *Staphylococcus aureus*. During the last weeks of her life, the liver could be felt. In the final stage, it reached half way between the xiphoid process and the level of the navel in the median line. It had a relatively solid border. Some superficial lymph nodes of the size of grains could be felt in the inguinal and axillary regions. On the wall of the chest along the left axillary line, a solid gland the size of a bean appeared, which was excised for histologic examination.

The general condition of the child became gradually worse with eruptions of new furuncles, inflammatory infiltrations, violent stomatomycosis and high fever. She finally died at the age of 5 months and 22 days.

Special Examinations

Blood examinations have been summarized in Table I (p. 15) and Fig. 3 (p. 13).

Bone Marrow Examinations (N. G. Nordenson, M.D.)

March 17, 1949: Sternal punctate: Cell-rich preparation. In myelopoiesis, a block in the maturation process after promyelocytes was observed. Signs of toxic maturation disturbance were noted. The eosinophilic cells were slightly increased. Erythropoiesis was moderately hyperplastic with normal maturation. The megakaryocytes were normal.

Diagnosis: Granulocytopenia. Grave changes. Nothing definitely specific in the picture.

April 1, 1949: Sternal punctate: Scanty cell preparation. Myelopoiesis was only represented by a few myeloblasts. Erythropoiesis was sparse. The megakaryocytes were normal. The reticulum was slightly hyperplastic.

Diagnosis: Agranulocytosis. Same picture as in the previous examination.

April 21, 1949: Sternal punctate: Relatively cell-rich preparation. Myelopoiesis was strongly inhibited with a block after myeloblasts-promyelocytes. Erythropoiesis was largely normal. The megakaryocytes were very rare. The reticulum was greatly hyperplastic.

Diagnosis: Granulocytopenia. The cells were now more immature with an increase in myeloblasts, of which many had atypical forms of the paramyeloblast type. The findings suggested an acute leukemia.

May 11, 1949: Sternal punctate: Scanty cell preparation. In principal, the same changes as in previous punctures were noted. The picture was dominated by reticular and mononuclear cells which partly resembled micro-myeloblasts.

Diagnosis: Granulopenia with some suspicion of acute leukemia.

May 28, 1949: Sternal punctate: Cell-rich preparation. In myelopoiesis, a maturation inhibition was noted with a block in the maturation process after myelocytes. The findings suggested toxic maturation disturbances. In erythropoiesis, signs of maturation disturbance (polychromasia) were observed. The megakaryocytes were rare. The reticulum was strongly hyperplastic.

Diagnosis: Strongly reactive bone marrow. Granulopenia. Systemic disease?

June 13 1949: Sternal punctate: Cell-rich preparation. In myelopoiesis, the strong maturation inhibition with block in the maturation process after myelocytes still dominated. Monocytoid reticular cells were rather numerous. In erythropoiesis, a strong maturation disturbance was noted. The megakaryocytes were normal. The reticulum was strongly hyperplastic with an increase in lymphoid cells.

Diagnosis: Granulopenia. Systemic disease? Worse condition since the previous examination.

TABLE I

Case A9. Blood examinations.

Date	Hemoglobin %	Red blood cells million pr mm ³	White blood cells pr mm ³	Stab cells %	Segmented neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Unspecified cells %	Platelets pr mm ³	Sedimentation rate
18/3	72	4.22	6,400	—	—	—	—	57.5	41.5	1	260,000	56
20/3			9,600	—	—	0.5	—	55	43.5	1		
21/3			6,200	—	—	5.5	—	56.5	37.5	0.5		
22/3			7,000	—	—	2	—	52	45	1		
23/3	75	4.02	7,400	—	—	0.5	—	43.5	56	—		33
24/3			9,200	—	0.5	1	—	38.5	57	3		
25/3			10,100	—	—	1	—	54	44	1		
26/3			10,000	—	—	—	—	49	45	6		
27/3			8,400	—	1	1	—	60	33	5		
28/3			10,000	—	0.5	0.5	—	43	54	2		
29/3	84	4.47	3,700	—	1	1	1	47	49	1	260,000	38
30/3	110		8,100	—	—	—	—	60	39	1		
31/3			6,500	—	—	1	1	65	32	1		
1/4			4,600	—	—	—	—	67	31	2		
2/4	107		6,200	—	1	2	—	53	44	—		
3/4			5,600	—	1	1	1	62	35	—		
4/4			8,100	—	—	3	1	60	34	1		
5/4			7,800	—	—	2	1	64	31	1		16
6/4	97		6,400	—	1	2	1	62	33	1		
9/4	100		11,000	—	—	—	—	52	47	1		
13/4	93	4.68	6,500	—	—	2	—	40	58	—	393,000	26
16/4			9,300	—	—	1	1	50	46	1		
20/4	84		11,700	—	—	1	0.5	71.5	26.5	0.5		52
23/4			11,900	—	—	3	—	57	38	1		
27/4	87		10,700	—	—	3	—	50	46	1		53
29/4												
30/4			8,500	—	1	3	—	40	55	1		
4/5	76	4.26	9,000	—	—	6	—	38	54	1		
7/5	98	4.90	10,100	—	—	5	—	35	60	—		
11/5	63	3.54	8,900	—	1	2	—	42	53	1		
14/5	68	3.90	4,500	—	—	1	—	61	34	1		
15/5			8,100									
21/5	72	3.64	10,200	1	12	—	—	84	3	—	393,000	
24/5	71	3.39	6,800	1	10	—	—	88	1	—		
27/5	75	3.48	6,600									61
3/6	73		3,100	—	5	—	—	70	25	—		68
13/6	72	2.10	5,600	—	—	—	—	78	22	—		60
17/6	70	3.20	7,000	—	1	1	—	70	28	—		
25/6	70	2.78	6,100	—	—	—	—	68	40	—		

23/3 Protrombin index 63 Bleeding time: 2 min 35 sec

2/4 „ „ 92 Clotting time: 3 min 30 sec

Bleeding time: 1 min 30 sec

29/4 „ „ 89 Clotting time: 9 min 55 sec

June 25 1949: Sternal punctate: Relatively scanty cell preparation. The picture was dominated by a strongly hyperplastic reticulum with monocytoid elements. The myelo-erythropoiesis was extremely sparse.

Diagnosis: Granulopenia. Systemic disease?

X-ray examination of heart and lungs. May 2, 1949: Heart of normal size. In the lungs, intensified striation in hilus medially towards the bases and parenchymal condensation basally behind the heart.

X-ray examination of skeleton. May 2, 12, 23 and June 22, 1949: No pathologic structural changes.

Ophthalmological examinations. June 22, 1949: Sclerae somewhat bluish, but not definitely pathologic. Otherwise normal conditions.

Electrocardiogram. May 8, 1949: Normal.

Lumbar punctate. April 22, 1949: Normal conditions. No growth upon culturing the spinal fluid.

Bacteriologic Examinations

Blood culture, March 17, 1949: No growth. Material from pus blister, March 17, 1949: Growth of Gram-positive cocci (Staphylococci). March 19, 1949: Rich growth of pathogenic strain of Staphylococcus aureus. Penicillin and sulfa resistant. Streptomycin sensitive.

Throat culture, May 21, 1949: Growth of α -streptococci: Sulfa and streptomycin resistant. Penicillin sensitive. Staphylococcus aureus: Sulfa resistant. Penicillin and streptomycin sensitive. Coliform bacteria: Sulfa, penicillin and streptomycin resistant.

Nose culture, May 21, 1949: Growth of α -streptococci: Sulfa and penicillin sensitive. Streptomycin resistant. Staphylococcus albus: Sulfa resistant. Penicillin and streptomycin sensitive.

Secretion from the right ear, May 21, 1949: Growth of Bacillus pyocyaneus. Sulfa, penicillin and streptomycin resistant.

Secretion from necrotic sore in the scalp, May 21, 1949: Growth of α -streptococcus. Sulfa and streptomycin resistant. Penicillin sensitive. Staphylococcus aureus: Sulfa resistant. Penicillin and streptomycin sensitive.

Secretion from necrotic sore on the back, May 21, 1949: Growth of Staphylococcus aureus: Sulfa resistant. Penicillin and streptomycin sensitive.

Pus from abscess, May 23, 1949: Growth of Staphylococcus aureus: Sulfa and penicillin resistant. Streptomycin sensitive.

Pathologic-Anatomic Examinations (Fredrik Wahlgren, M.D.)

Histological examination of a *lymph node* from upper part of thorax (June 2, 1949): In the examined lymph node, which was the size of a coffee bean, the normal architecture was erased. This appeared due to very large growth centers consisting of pathological cells in the lymph follicles. The different follicles were separated only by narrow streaks of lymphatic tissue with lymphocytes and somewhat enlarged reticular cells. In addition, there were a few eosinophilic leucocytes in the tissue, but otherwise no pathological cell elements were seen. The vessels of the lymph node had markedly enlarged endothelial cells. The sinus appeared compressed and empty. The lymph node was inbedded in a thick capsule of cell-poor, dense connective tissue in which there were scattered lymphocytes as well as some large mononuclear cells, which, in their structure, most closely resembled the cells in the germinal centers.

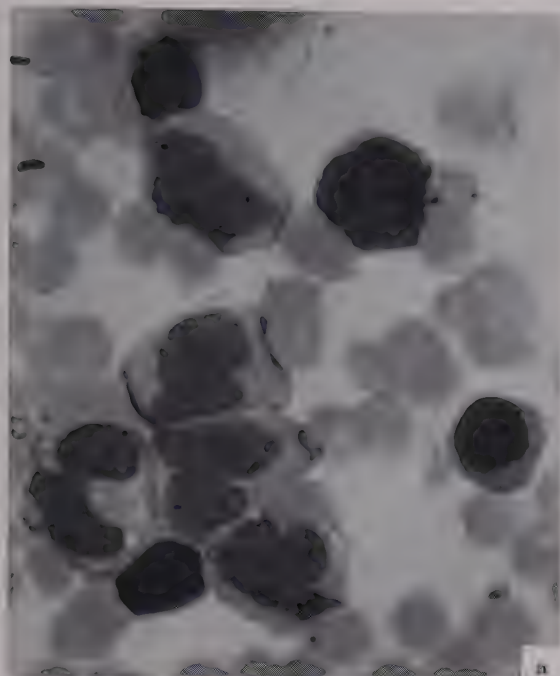
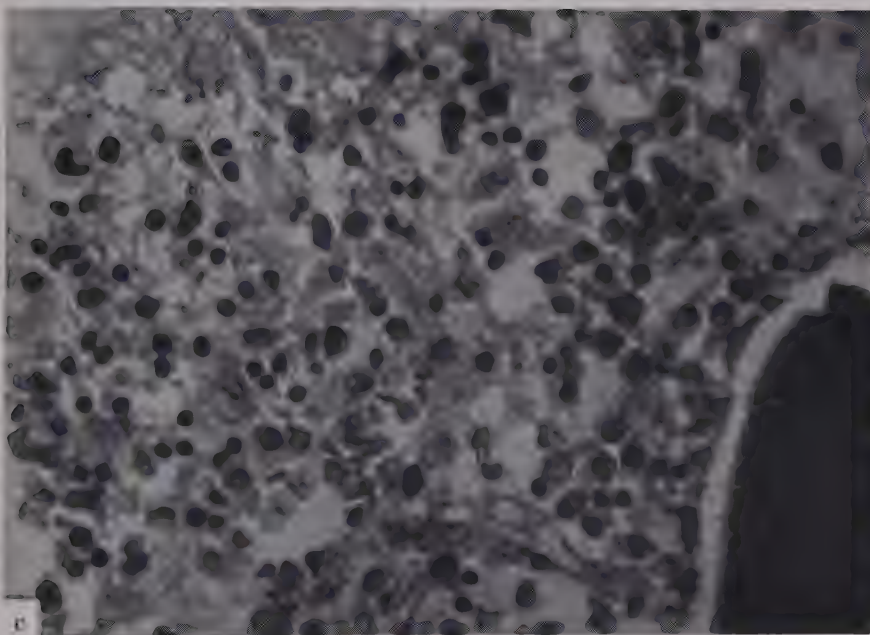
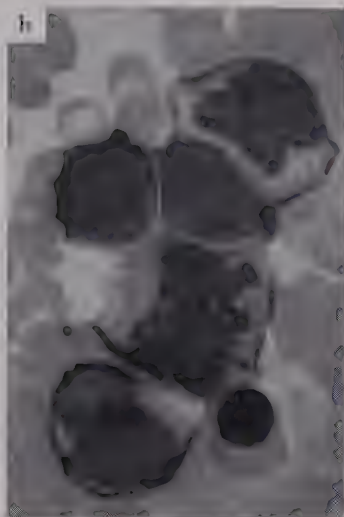


Fig. 4. Case A9. *a* and *b*, Microphotographs of bone marrow smears. *c*, Bone marrow (autopsy specimen). Predominance of immature red cell forms.



Autopsy

June 29, 1949: A child of ordinary size for its age with extremely sparse, slack flesh and pale grey skin color. Weight 4600 g. On the left side of the skull were a few brownish-violet papules in the skin, scarcely the size of hemp-seeds. Over the left kidney region were two large, irregular, dirty-brown-violet skin infiltrations, partly covered by yellowish-brown, dried crusts.

The brain and its membranes showed no changes. The bones of the skull were also normal. The hypophysis was of normal size.

The thymus was strongly reduced in size, and consisted of a thin, reddish-grey disk.

The pericardium contained a small amount of clear fluid. The heart and the large vessels had no malformations or other changes. The heart muscle was a pale reddish-brown.

Both lungs were free in the pleurae, which did not contain any fluid. The lung parenchyma was fully air-carrying and gave up a small amount of foamy, clear fluid from the surface of incision. In the trachea and bronchi, the mucous membrane was pale with some slimy, foamy coating.

In the posterior mediastinum, the lymph nodes were, at most, the size of coffee beans with dark greyish-red incision surfaces.

In the throat and upper part of the esophagus, the mucous membrane was pale grey with a few greyish-yellow, grainy coverings of the size of a hemp seed. From the upper chest aperture down to cardia, the esophagus was strongly dilatated in a funnel-shaped manner. Its mucous membrane was changed into dirty-greyish-yellow, grainy masses.

There was no free fluid in the abdominal cavity. The mucous membrane of the stomach was pale grey and without changes. The mucous membrane of the small intestines was also normal. There was no enlargement in the lymphoid system. On the ileocecal valve, there was a small, dirty-greyish-brown, grainy coating. The mucous membrane of the colon was pale and normal. The liver was of normal size and consistency, and had a pale reddish-brown incision surface which did not show any obvious architecture. Gall passages, the pancreas, and the portal vein were normal. The spleen was of normal size and consistency and had a dark red, even incision surface. Weight: 10 g. The adrenals were of normal size. The cortex was thin and rich in lipoid material. The medulla was pale greyish-brown. The kidneys were of normal size. They were pale, but otherwise normal. The urinary passages were normal. The normal curvature of the spine was completely straightened out. The bone tissue was dark red in the vertebrae and ribs.

Histological Examinations

Within the examined parts of the *lungs*, the alveoli were mostly filled with air, but occasionally contained a small amount of serous fluid or red blood corpuscles. The septae of the alveoli were very wide and rich in cells, with the vessels rather heavily filled with blood. There was also an accumulation of lymphocytoid cells and an enlargement of the reticular cells. There was no sign of giant cells or necrosis. The bronchi occasionally contained a small amount of mucus. The mucous membrane, as well as the wall, showed no obvious changes. In the mucous membrane of the *esophagus*, there were many ulcerations covered with necrotic masses with large amounts of fungi (*Oidium*). Around the ulcerations, the wall showed rather pronounced inflammatory changes.

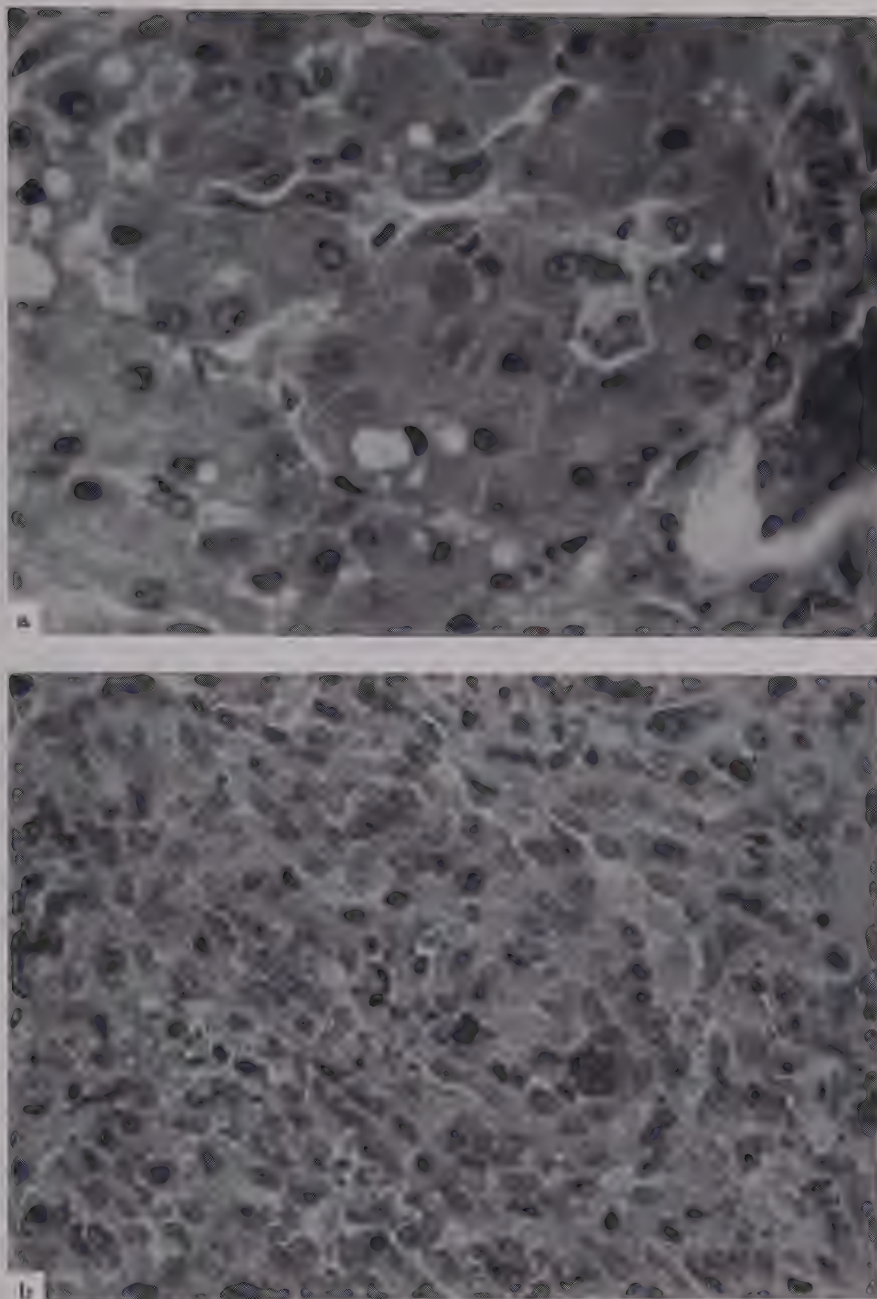


Fig. 5. Case 10. Autopsy specimens. *a*, Liver: Swelling of Kupfer cells and signs of fatty degeneration. *b*, Spleen: Swelling of sino-endothelial cells.

The *thyroid*, as well as the musculature of the neck, was histologically normal. In the *tonsils*, the lymphoid tissue was hyperplastic with enlarged reticular cells. There were no prominent germinal centers and no pathological cell elements were observed.

The follicles of the *spleen* (Fig. 5b, p. 19) were very small and lacked germinal centers. The pulp was devoid of blood and rather rich in cells. There was some enlargement of the sino-endothelial cells, but the pulp cells proper did not appear particularly enlarged. The pulp contained a very large amount of blackish-brown iron pigment. No pathological cell elements were observed.

In sections of the *small intestines*, an ulceration was found which, in some parts, penetrated to the outermost muscle layer. The base of the ulceration consisted of necrotic tissue which, at the bottom, changed into granulation tissue containing lymphocytes, plasma cells and fibroblasts. No giant cells were observed. The outer layers of the wall were edematously loosened and rather rich in inflammatory cells. In the serosa, fibroblast regeneration was observed.

The *lymph nodes of the mesentery* showed no gross changes. The tissue was rich in blood but it had, in general, a normal architecture. Possibly the lymph sinus contained a somewhat increased number of enlarged desquamated endothelial cells. Otherwise, no pathological cell elements were noted. The vessels and nerves of the mesentery appeared normal.

The acinous architecture of the *liver* (Fig 5a, p. 19) was rather distinct. The liver-parenchymal cells showed signs of fine-dropped fattification. The intra-acinous capillaries were collapsed and poor in blood. Quite a number of the Kupffer astral cells were enlarged. No signs of phagocytosis could be observed within them. No increase in periportal connective tissue was observed. Many Kupffer cells contained rather large amounts of brown iron pigment. Gall passages and vessels were normal. The *pancreas* did not show any noticeable histological changes.

In the *adrenals*, the architecture appeared normal. The tissue was rather rich in lipid material. The medulla contained large amounts of blood in which there were also numerous phagocytes containing iron pigment.

The *bone marrow* (Fig. 4c p. 17) was very poor in cells and appeared to contain mainly early precursors of the red blood cells. There were very few cells belonging to the leucocyte series. Megakaryocytes appeared in approximately normal numbers. The reticular cells were distinctly enlarged.

Epicritic Summary

A girl fell ill when three weeks old with an infection of the upper respiratory tract, which was complicated by otitis and mastoiditis. The latter was radically operated, but showed a poor tendency to heal. Multiple furuncles and abscesses appeared in the skin. She got high fever and her general condition worsened. The peripheral blood showed agranulocytosis. A maturation block at the promyelocyte-myelocyte stage was found in the bone marrow. The continued course of the disease was, above all, characterized by recurring skin afflictions. These could be counteracted by different antibiotics, but recurred when the treatment was discontinued. Her general condition became gradually worse. Finally, she became cachectic and died at

the age of six months. During the whole course of the disease, she displayed a pronounced granulocytopenia or agranulocytosis.

The clinical history, the blood picture, the intravital studies of the bone marrow, and the results of the post-mortem histologic examination justify the diagnosis of agranulocytosis. No findings indicated that this agranulocytosis should have had an exogenous toxic or allergic origin.

CASE D1

Girl. Born March 12, 1948. Died June 17, 1948. Normal delivery. Weight at birth, 2810 g. She was breast-fed for two months. At the age of three weeks, a bluish-red infiltration appeared on one popliteal region. The girl became fussy, cried a great deal, and ate poorly. At the age of one month, she was taken to the surgical ward in Gällivare Hospital. She was then highly febrile and generally affected. On the one buttock, on the right popliteal region, on the right shoulder, on the neck and behind the one ear were necrotic sores, and in the face and on the chest were some bluish-red infiltrations. She was treated with penicillin and became free of fever. The sores began to heal and she was discharged from the hospital. Two weeks later, an incision of a furuncle on the right popliteal region was made. After an additional three weeks, she got high fever again. She also vomited and had diarrhea. She was taken to the medical ward of the same hospital.

Status June 12, 1948: The general condition was considerably affected. She was pale and had toxicosis. No dyspnea. Fontanel normal. The larynx was considerably inflamed. A few rhonchi could be heard on the lungs. Liver, spleen and superficial lymph nodes could not be felt. She died June 17, 1948 when 3 months and 5 days old.

Special Examinations

Blood Examination, June 13, 1948: Hemoglobin 44 per cent; red blood cells 2.3 million per cu.mm.; white blood cells 800 per cu.mm. Differential count: myelocytes 14 per cent; metamyelocytes 49 per cent; lymphocytes 37 per cent (?). Thrombocytes 590,000 per cu.mm. Prothrombin index 72 per cent. SR 41 mm/1 hr. Blood groups: O Rh +. Mother: O Rh +. June 14, 1948: White blood cells 750 per cu.mm. Differential count: myelocytes 3 per cent; metamyelocytes 1 per cent; lymphocytes 96 per cent. June 15, 1948: White blood cells 1600 per cu.mm. June 16, 1948: White blood cells 1800 per cu.mm. June 17, 1948: It was noted in the records: The white blood corpuscles have increased somewhat in number, but not a single granulated cell can be seen.

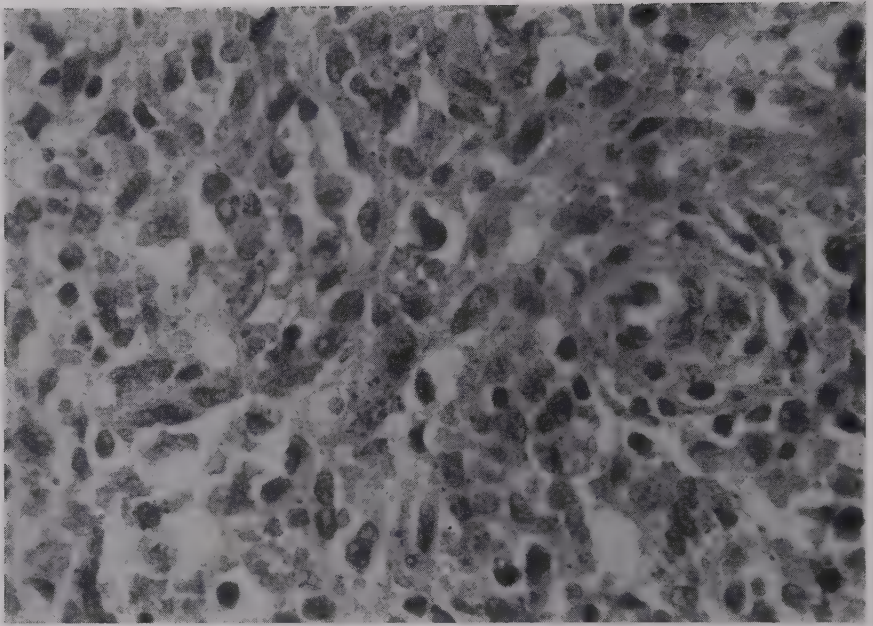


Fig. 6. Case D1. Autopsy specimen. Spleen: Swelling of sino-endothelial cells.

Autopsy

The heart was normal. The pericardium contained large amounts of blood-colored fluid. Fibrin deposits were found on the visceral pericardium. The lungs adhered to the pleura, and could only be freed from it with difficulty. The lungs felt as if they were penetrated by small infiltrations, probably bronchial pneumonias. There was no free fluid or fibrin deposits in the abdominal cavity. The intestines were meteoristic. The liver was pale, the spleen small and the kidneys normal. The renal pelvi contained a thin, pus-like fluid.

Histological Examination (Fredrik Wahlgren, M.D.)

The follicles of the *spleen* (Fig. 6) were small and lacked germinal centers. They showed neither sclerosis nor other regressive changes. The pulp was moderately rich in blood. The sinus contained relatively few red blood cells. The sino-endothelial cells appeared somewhat enlarged. The pulp was rather rich in cells, which were enlarged. In addition, it contained a few leucocytes, among which were also a few eosinophils. The pulp appeared to be richer than usual in collagen, but silver staining did not demonstrate any obvious increase of the reticulum. There were some local centers of destruction in the spleen tissue.

The acinous architecture of the *liver* (Fig. 7b) appeared well preserved. The liver parenchymal cells showed moderately pronounced large-dropped fattification. The intraacinous capillaries were moderately wide and contained little blood. The Kupffer astral cells were enlarged. The periportal connective tissue seemed somewhat more abundant than usual and contained a few mononuclear cells. Gall passages and vessels were normal.

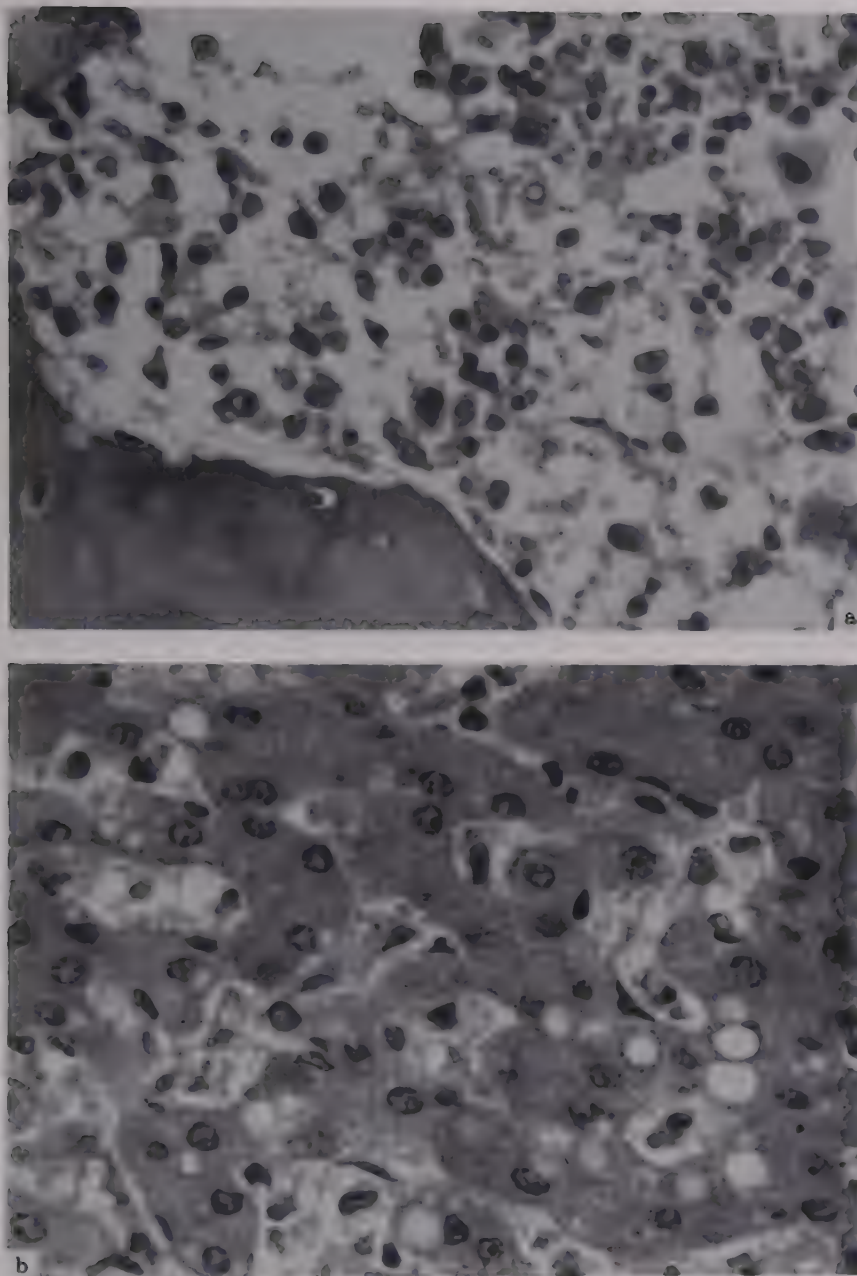


Fig. 7. Case D1. Autopsy specimens. *a*, Bone marrow: Poverty of cells and predominance of immature red cell forms. *b*, Liver: Swelling of Kupfer cells and signs of fatty degeneration.

The *bone tissue* of the ribs (Fig. 7a, p. 23) did not show any changes. The bone marrow was relatively poor in cells. It appeared to contain predominately precursors of erythrocytes. There were a few pathological cell elements which were interpreted as megaloblasts. The reticular cells were distinctly enlarged. The megakaryocytes were few in number and displayed pathological forms with peculiar, chromatin-rich nuclei that showed signs of disintegration.

Epicritic Summary

A girl fell ill at the age of three weeks with a phlegmonous infiltration on the one popliteal region. When taken to the hospital at the age of one month, she was highly febrile and had multiple septic infiltrations and necrotic sores of the skin. She was treated with penicillin and incisions. The septic skin afflictions recurred several times, and the child was treated ambulantly with incisions and penicillin. At the age of three months, she was again taken to the hospital. She was then highly febrile and moribund. The peripheral blood showed leucopenia, agranulocytosis and anemia. She died after a few days at the age of 3 months.

The clinical history, the blood picture and the results of the pathologic-anatomic examinations justify the diagnosis of agranulocytosis. There are no indications that this agranulocytosis should have had an exogenous toxic or allergic origin.

CASE T9

Girl. Born July 21, 1953. Died September 3, 1953. Normal delivery. Weight at birth, 3200 g. Vaccinated against tuberculosis. She was breast-fed. At the age of five days, a pea-sized, reddish-blue, elevated efflorescence appeared above the right ear. After a few days, pus mixed with blood was discharged. Penicillin ointment was prescribed. The sore continued to discharge. At the age of two weeks, she was taken to the children's ward of Gällivare Hospital and was treated there for ten days. Upon arrival, she was thin but otherwise in a good general state. She had a pronounced stomatomycosis with white deposits on the buccal mucosa and tongue. Within an area of 2×1 cm just above the navel, the skin was swollen and miscolored and bluish-red. In the scalp immediately above the right ear, she had an oval, bluish-red swelling about 1.5×2 cm in size. This bordered on a bluish miscolored skin area with a diameter of about $2\frac{1}{2}$ cm, where the skin appeared thin and partly eroded. A light red streak stretched down the swollen cheek. Close to the median line in the left fronto-parietal region, a pea-sized, cyanotic, furuncle-like formation could be seen. Her tempera-

ture was normal. The internal organs were normal. White blood cells 8100 per cu.mm. Differential count: segmented granulocytes 17 per cent; stab cells 8 per cent; eosinophils 0 per cent; basophils 0 per cent; lymphocytes 55 per cent; monocytes 20 per cent. She was treated with penicillin and the local skin changes regressed. The swelling above the right ear decreased. A skin infiltration in the cheek could be felt corresponding to the parotid gland. This infiltration still remained when she was discharged from the hospital. At a control examination three weeks later, the skin above the ear and on the cheek was normal. Small areas with folliculitis were scattered around the neck and in the right groin.

At a new control September 1, 1953, she had a large abscess on the right side of the neck and a smaller one in front of the right ear. She had scattered areas of folliculitis all over the body. She looked greyish-pale and sickly and had a temperature of 39.5°C. On the right side of the neck and in the shoulder region, she had large abscesses which had partly emptied themselves. Around the neck and in the armpit there were a great number of small areas of folliculitis. Such areas were also found in skin folds on other parts of the body. She had a red spot and swelling in front of the right ear. Her weight was 2960 g. Blood: Hemoglobin 97 per cent; erythrocytes 5 million; white cells 10,800 per cu.mm. Differential count: segmented granulocytes 4 per cent; stab cells 0 per cent; eosinophils 0 per cent; basophils 0 per cent; lymphocytes 80 per cent; monocytes 16 per cent. This preparation was later checked by N. G. Nordenson, M.D., with the following result: Segmented granulocytes 0.5 per cent; lymphocytes 26.5 per cent; monocytes 73 per cent. Dr. Nordenson's diagnosis was agranulocytosis with mainly monocytous reaction. The monocytes did not appear directly pathological.

She was treated with penicillin. A few days later, a sudden change for the worse took place, and she died.

Pathologic-Anatomic Examination (Fredrik Wahlgren, M.D.)

The acinous architecture of the *liver* (Fig. 8a) could not be distinctly seen. The liver cells were enlarged. Large spaces were seen in the cytoplasm, particularly in the cells of the peripheral acini, probably resulting from dissolution of fat. The capillaries between the sini were compressed and empty. In the central parts of the acini, however, a small number of red or white corpuscles could be seen. The majority of the Kupffer astral cells were enlarged. Some of them contained a fine-grained, brown pigment, probably an iron pigment. The gall bladder and ducts were normal. The follicles of the *spleen* (Fig. 8b, p. 26) were small and few in number. They lacked germinal centers. The pulp was rather poor in blood, but rich in cells. The reticulum was distinctly increased. In the pulp, numerous large phagocytes carrying iron pigment were found. The vessels and the trabecular system appeared essentially normal. The *thymus* was remarkably poor in cells and contained very few lymphocytes. The reticular cells were enlarged. Only few Hassal's corpuscles were found. A *lymph node*, the size of a

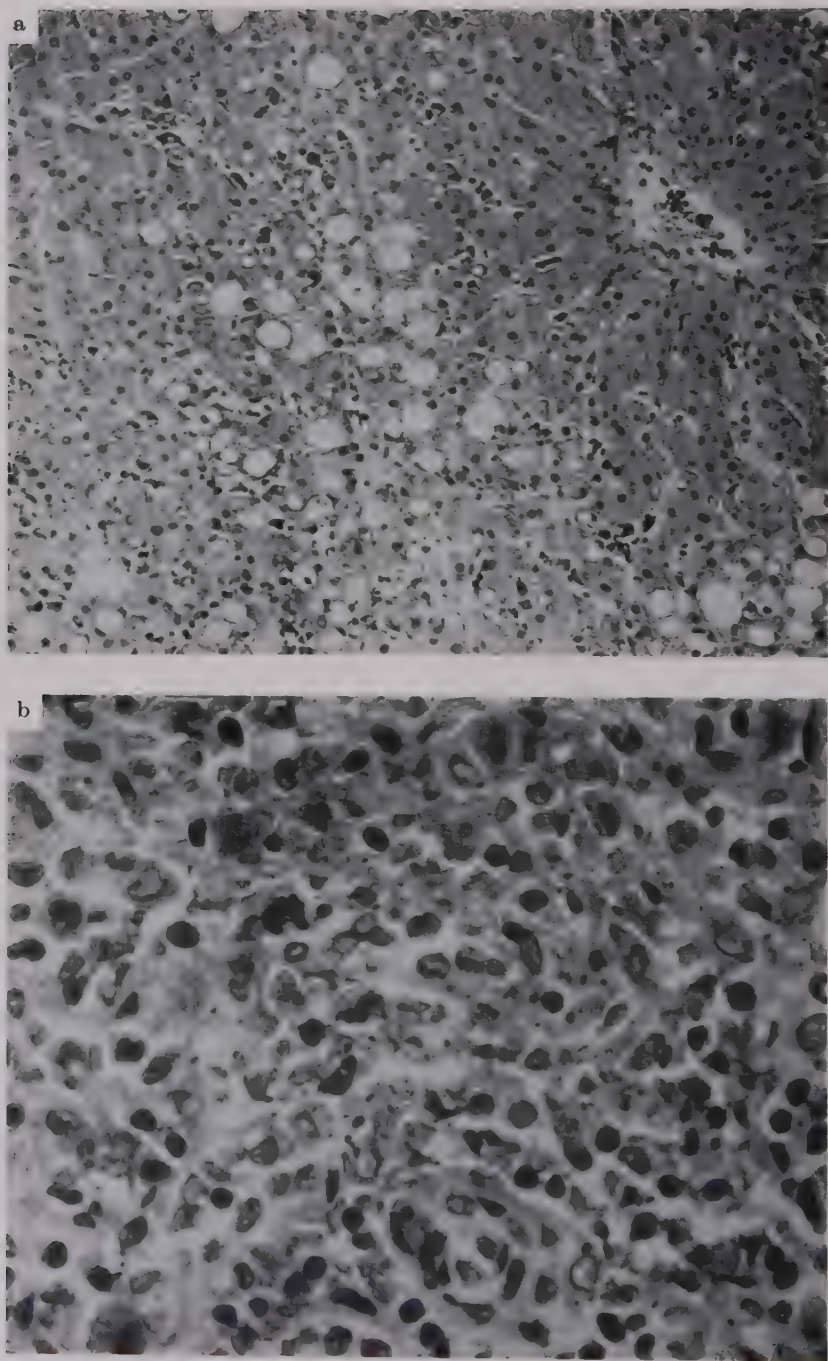


Fig. 8. Case T9. Autopsy specimens. *a*, Liver: Signs of fatty degeneration. *b*, Spleen: Swelling of the sino-endothelial cells.

hemp seed, showed a distinct enlargement of the sinus endothelium and an increase of the reticulum. The lymphocytes were considerably fewer than normal. Scattered eosinophils and phagocytes carrying an iron pigment could be seen. The *adrenal glands* were normal. The *bone marrow* was rather poor in cells, but, as far as could be judged without making a detailed counting of the cells, the distribution of the different cell types appeared normal. No obvious enlargement of the reticular cells could be observed.

The major findings in this case were an enlargement of the endothelial cells and an increase of the reticulum in the spleen and the lymph nodes. This indicates an increased breakdown of erythrocytes, but does not explain its cause. The reduction in the number of lymphocytes in different organs was remarkable.

Epicritic Summary

A girl fell ill at the age of three weeks with a phlegmon in the scalp. She had no fever. Blood examinations showed granulocytopenia and a strong shift to the left within the peripheral white blood cells. She was treated with penicillin and improved. After another month, she became gravely ill, had high fever and a few large abscesses on the neck. Blood examination showed agranulocytosis. She was again treated with penicillin, but died after a few days, at the age of two months.

The clinical course, the blood examinations and the result of the pathologic-anatomic examination justify the diagnosis of agranulocytosis. There were no indications that this agranulocytosis should have had an exogenous toxic or allergic origin.

CASE R4

Boy. Born June 15, 1951. Weight at birth, 3200 g. He was breast-fed for 4 months, after which a supplement of gruel was given and, after the age of 7 months, he got a mixed diet. When he was $2\frac{1}{2}$ months old, an abscess in the one gluteal region was treated by a physician with an incision and penicillin. The wound healed in two weeks. After an additional two weeks, another abscess on the right side of the trunk was treated with penicillin. At the age of four months, he got another abscess the size of a walnut on the scalp and several small furuncles on the neck. At this time, he had also high fever. He was taken to the hospital in Kalix, received aureomycin, and was discharged as recovered. At the age of seven months, a furuncle appeared in the right auditory canal. He was again taken to the Kalix Hospital and received aureomycin for two weeks. Upon returning home, he was pale, fussy, perspired much, and had occasional fever. One month later, new abscesses in the scalp appeared. He was again treated with aureomycin at home. His temperature became normal and the abscesses healed.

At the age of ten months, he had fever again and new abscesses on the scalp. He was taken to the pediatric clinic in Boden. Upon admission, he was febrile and had a dirty-pale skin color. He was thin and his tonus and turgor were decreased. There were several furuncles and abscesses of varying size on the scalp and neck, the largest one being 5×5 cm (Fig. 9). In the center of this abscess there was a bluish-red necrotic spot, covered by a crust. On the neck, there was an abscess the size of a walnut in the process of drying. There were radiate scars from earlier abscesses in several places on the scalp, neck, gluteal regions and the trunk. On the neck were several bean-sized, firm adenites and on both groins, pea-sized adenites. The liver was palpable, but not noticeable enlarged. The spleen could not be felt. Other internal organs appeared normal upon physical examination.

The white blood cells showed an almost complete agranulocytosis. A bacteriological blood culture was negative. Cultures from abscesses gave growth of a pathogenic strain of *Staphylococcus aureus*. Antistreptolysin titer 22 units per ml. Antistaphylosin titer 28 units per ml. He was treated with penicillin and chloromycetin, and became free of fever in ten days. On April 27, 1952, he was referred to the pediatric clinic of the University Hospital, Uppsala, for continued treatment and special examinations. He was under treatment and observation there until October 28, 1952.

During the whole time, he had complete agranulocytosis (Fig. 10, p. 30). The bone marrow showed a block in maturation at the myelocyte stage. X-ray examinations of the skeleton, heart and lungs showed normal conditions. The total blood protein quantity was normal, but there was a great increase in γ -globulin (42 per cent). Wassermann reaction in blood was negative. Bacteriological cultures from abscesses gave growth of *Staphylococcus aureus*.

Attempts to improve the bone marrow with di-menformone, folacin, pernaemon forte with vitamine B₁₂, and cortisone were of no effect.

Cell culture experiments with bone marrow were performed (cf. p. 34). Activation and maturation of the cells were obtained when cysteine or normal serum was added. As a therapeutic trial, cysteine was given orally, subcutaneously and as intravenous drip, but without effect.

During the entire stay in the hospital, he obtained, in different periods, large doses of chloromycetin, penicillin, elkodin, and streptomycin. On several occasions, he had remittent fever, as a rule without other objective findings. On one occasion, he had a severe balanitis, and, on another occasion, an abscess following an injection of pernaemon.

The general development of the boy was retarded. He increased but slowly in weight (7140–8750 g.). During the entire stay in the hospital, he had temper tantrums almost daily, and often several times a day. The

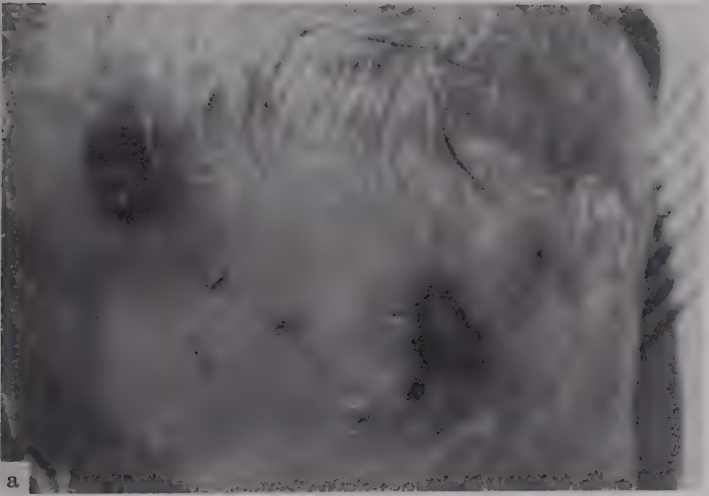
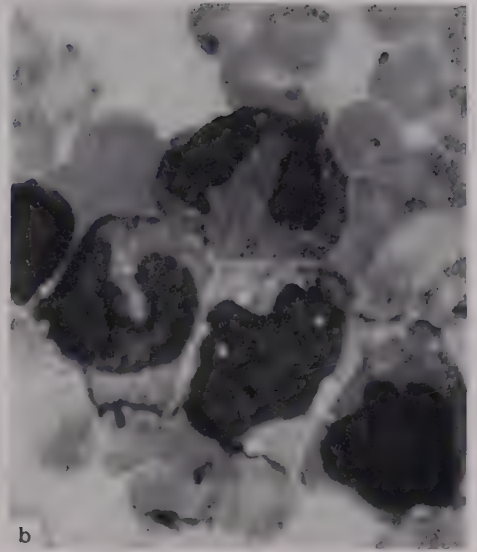


Fig. 9. Case R4. *a*, Abscesses of the neck. *b*, Microphotograph of bone marrow smear.

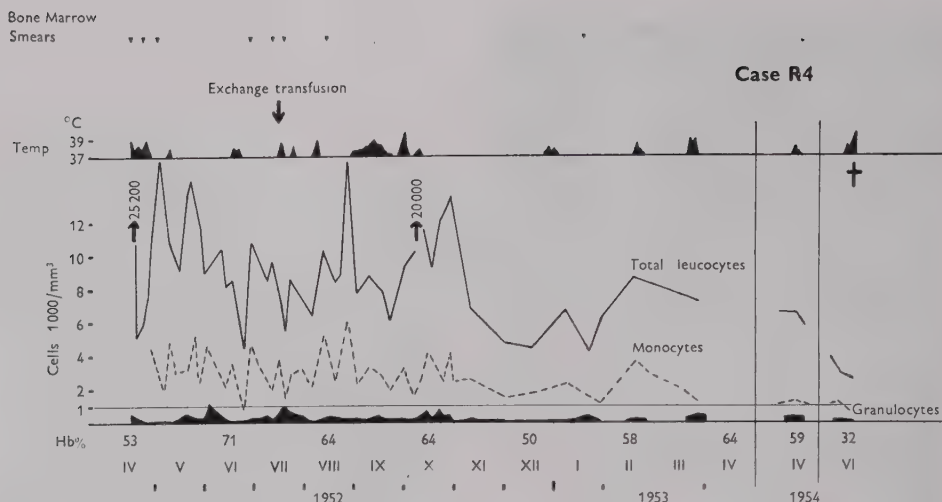


Fig. 10. Case R4. Graphical representation of the blood values during the course of illness.

psychic development otherwise appeared relatively normal. His electroencephalogram was normal.

After discharge from the hospital in Uppsala (October 28, 1952), he was treated in his home. During 1953 and 1954, he had numerous infections of different kinds, anginas, septic-necrotic sores in the mouth, cutaneous abscesses and bronchial pneumonias. He was treated with antibiotics by the district physician and, on several occasions, at the Children's Hospital in Boden. The blood examinations constantly showed agranulocytosis. His general condition gradually became worse. Cramp attacks of a more genuine grand mal character were observed occasionally.

In July 1954, he was again hospitalized with extensive bronchial pneumonias, and died after a few days on July 28, 1954. He was then three years and one month old.

Special Examinations

Blood examinations have been summarized in Table II and Fig. 10.

Bone Marrow Examinations

April 16, 1952: (N. G. Nordenson, M.D.) Sternal punctate: Cell-rich preparation. Myelopoiesis was totally blocked after promyelocytes. In addition, a maturation disturbance in erythropoiesis was observed. The red blood cells showed a marked anisocytosis. The megakaryocytes were normal. The reticulum was strongly hyperplastic with an increase in lymphoid elements.

Diagnosis: Probably an agranular reaction. Suspected leukemia.

TABLE II

Case R4. Blood examinations.

Date	Hemoglobin %	Red blood cells million per mm ³	Reticulocytes per 1 000	White blood cells per mm ³	Stab cells %	Segmented neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Plasma cells %	Platelets per mm ³	Sedimentation rate
1952													
16/4	53	3.46		25,200	0.5	1.5	—	—	56	42	—	227,000	61
19/4	49	3.26		5,100	—	—	—	—	93	7	—		
23/4		3.86		6,000									65
25/4	55	3.84		7,700	—	—	—	0.5	95.5		—		
28/4	60	3.75	30	11,800	—	—	0.5	—	58	38	3.5	246,000	44
2/5	63	3.40		16,200									57
3/5					—	0.5	—	1	50	48	0.5		
6/5	57	3.75		6,700	—	0.5	—	1.5	66.5	29	2.5		59
9/5	53	3.10		10,900	—	2	—	1	48	49	—		53
13/5	52	3.60		10,200	—	—	1	1	68	30	—		52
15/5	53	3.47	12	9,200	—	—	2	1	61	36	—		58
17/5			62										
20/5	52	3.56	56	13,600	—	—	2.5	1.5	72.5	23.5	—		38
22/5	56	3.49	32	14,600									40
24/5			20	13,100	—	—	5	1.5	54	39.5	—		
27/5	58	3.56	18	11,800	0.5	0.5	1	0.5	77.5	20	—		30
29/5			10										
30/5	59	3.48		9,000	—	0.5	2	0.5	75	22	—		26
1/6			6										
3/6	62	3.61	11	19,000	—	—	5	1	69.5	24.5	—		31
10/6	58	3.02	4	10,400									30
13/6	58	3.75	10	8,200	—	0.5	2	—	72	25.5	—		39
17/6	71	4.06	10	8,300	0.5	1	—	0.5	55.5	42.5	—		
24/6	56	3.52	22	4,500	—	—	1	0.5	80.5	17	1		32
28/6	56	3.82	50	10,600	—	0.5	0.5	1.5	53	44.5	—		31
1/7	61	4.02	24	10,600	—	1	2	—	68.5	28.5	—		42
3/7	59	3.92	52	9,600	—	2	1.5	—	59.5	37	—		
8/7	54	3.88	32	8,600	—	3	1.5	1	62	32	—		24
11/7	57	3.76	12	9,500	—	—	2.5	1	74.5	21	1		35
13/7			14										
15/7	61	4.24	24	23,300	0.5	—	2	—	81	16.5	—		16
16/7	exchange transfusion												
17/7	73	3.30	42	7,000	1.5	3	9	—	78.5	8	—	302,000	7
19/7	70	3.32		5,600	—	—	16.5	1.5	52.5	26	3		
22/7	67	3.57	24	8,600	—	0.5	7.5	—	56.5	34.5	1		38
29/7	65	3.28	26	7,300	—	1.5	2	2	45	44.5	5		40

(cont.)

(Table II, cont.)

Date	Hemoglobin %	Red blood cells million per mm ³	Reticulocytes per 1000	White blood cells per mm ³	Stab cells %	Segmented neutrophils	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Plasma cells %	Platelets per mm ³	Sedimentation rate
5/8	65	3.20		6,300	0.5	—	1	1	61	34.5	2		36
12/8	64	3.78	10	11,400	—	0.5	2	0.5	51	46	—		43
19/8	64	3.86	22	8,300	—	1	1.5	0.5	66.5	30.5	—		27
22/8	65	3.56	24	8,900	—	—	1.5	0.5	60	37.5	0.5		
26/8	64	3.25	26	15,800	—	—	—	1.5	60	38.5	—		36
2/9	62	3.20	26	7,900	—	1	2	1	66.5	28.5	1		43
9/9	52	2.53	18	8,800	0.5	1.5	—	1	59.5	37.5	—		53
16/9	53	2.80	14	8,000	—	1.5	1.5	2.5	56	37	1.5		34
22/9	54	3.08	28	6,200	—	1	—	3.5	58	30.5	1		27
30/9	57	3.26	20	9,300	—	—	—	1.5	62	36.5	—		
7/10	53	3.30	16	10,300	—	—	0.5	1	80.5	16	2		
10/10	59	3.15	30	15,500	—	—	—	—	84.5	15.5	—		34
14/10	54	3.41	30	18,600	—	—	3	1	74.5	21.5	—		26
17/10	64	3.50	20	9,300	—	1.5	1	0.5	65	32	—		34
21/10	62	3.28	8	20,000	—	—	1.5	2.5	81	15	—		28
24/10	63	3.62	18	11,900	—	0.5	0.5	1	78.5	19.5	—		26
28/10	65	3.30	10	13,600	—	—	3	0.5	63.5	32	1		42
30/10	49	3.00		5,100	—	0.5	2.5	0.5	46.5	50	—		
6/11	57												
10/11	54			6,900	—	3	1	—	57	38	—		
12/11	56												
19/11	55												
26/11	50												
1/12	47	3.06		4,900	1	0.5	—	—	66	32.5	—		
10/12	54												
17/12	50			4,500									
1953													
6/1	56												
7/1	54	3.52		6,700	1	1	—	—	63	35	—		
8/1					—	4	0.5	—	71.5	21	3	(Norden- son)	
14/1	58												
21/1	54	3.60		4,300	—	6	4	—	57	33	—		
28/1	63	3.84		6,200	—	0.5	1	0.5	78	20	—		
18/2	53	3.63		8,700									68
19/2			4	8,600	—	1	—	2	53	43	—	34,500	
26/3		3.12		7,300	—	4	1	2	75	18	—		73
8/4	64												

(cont.)

(Table II, cont.)

Date	Hemoglobin %	Red blood cells million per mm ³	Reticulocytes per 1000	White blood cells per mm ³	Stab cells %	Segmented neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Plasma cells %	Platelets per mm ³	Sedimentation rate
1954													
3/4	59	3.64		6,600	—	2	3	2	72	20	—		53
5/4			1									284,000	
13/6	39	2.49	1	5,000	1	1	—	—	75	22	—	196,000	63
14/6	38	2.48	6	4,300									
18/6	32	1.75		4,800	—	1	—	—	85	14	—		
22/6	29												

April 23, 1952. (N. G. Nordenson, M.D.) Relatively cell-rich preparation. The normal structure was completely erased, and the picture was dominated by monocytes, monocytoïds, lymphocytes (partly pathologic), plasma cells and reticulum cells.

Diagnosis: Agranular pattern, now less leukemic than at the previous examination.

May 2, 1952. Myelogram (Professor B. G. Vahlquist):

	Per cent
Myeloblasts	0.2
Promyelocytes.	—
Myelocytes: neutrophilic ¹	55.8
eosinophilic	2.0
basophilic	0.2
Metamyelocytes: neutrophilic	1.2
eosinophilic	0.8
basophilic	—
Stab cells: neutrophilic	0.4
eosinophilic	—
basophilic	—
Polymorphonuclear neutrophils	0.2
eosinophils	0.4
basophils	—
Normoblasts: acidophilic	2.4
basophilic.	9.4
Megakaryocytes	0.2
Reticuloendothelial cells	3.4
Ferrata cells.	—
Monocytes	1.0
Lymphocytes ²	19.8
Plasma cells.	5.4

¹ Partially large, vacuolated.
² Many atypical, resembling fibroblasts.

June 28, 1952. Tibia punctate (F. Nordbring, M.D.): Myelopoiesis was blocked approximately at the myelocyte stage. Singel cells matured as far as to stab nucleated cells could, however, be seen. The reticulum was hyperplastic. Erythropoiesis appeared retarded.

July 11, 1952. Sternal punctate (F. Nordbring, M.D.): The pattern was essentially the same. A few metamyelocytes, single stab nucleated cells and an occasional mature leucocyte could be seen. The lymphocytes were increased in number. Erythropoiesis appeared somewhat more active.

July 19, 1952. Sternal punctate (F. Nordbring, M.D.): On comparison with earlier preparations prior to an exchange blood transfusion on July 16, 1954, this preparation showed more stab nucleated and segmented nucleated cells; the latter were predominantly eosinophilic. Eosinophilic myelocytes and metamyelocytes were also numerous. Erythropoiesis appeared more active than before. Otherwise, lymphocytous and reticulous elements dominated as in earlier preparations. Several plasma cells were seen.

January 8, 1953. Sternal punctate (N. G. Nordenson, M.D.): The pattern was, in principle, the same as on previous occasions. A certain improvement appeared to have taken place, since the myelopoiesis showed more mature forms.

Diagnosis: Granylocytopenia. Reticulopathy.

April 10, 1954. Tibial punctate (N. G. Nordenson, M.D.): Cell-poor preparation. The pattern showed a dominance of lymphatic, monocytous, and reticulous elements.

Special Examination of the Bone Marrow including Cell Culture Experiments (C. Munk Plum, Ph.D., Copenhagen)¹

Bone marrow smear preparations: The bone marrow displayed a complete inhibition of myelopoiesis at the myelocyte stage. A number of more or less atypical mitoses could also be observed. Thus, some tetraploid mitoses and, probably as a consequence of this, atypical forms of nuclei were also found. The monocytes and especially the plasma cells appeared to be increased. Certain parts of the preparation resembled the findings in myelomatosis.

(1) Culture of the patient's bone marrow cells in his own serum: After the course of 24 hours with determinations at 0, 3, 6, 9 and 24 hours, it was found that, despite a slight increase in the number of immature precursors, the regeneration of erythrocytes calculated per normoblast was somewhat lower than normal, 4.23 compared to the normal of 5.39, i.e. 79 per cent of normal activity. No maturation of the myelopoietic cells could be observed.

(2) Culture of the patient's bone marrow cells in the serum of a normal individual: An increased activity of the normoblasts was found. The regeneration per normoblast was 4.79 with one normal serum and 4.84 with another. This regeneration is remarkable since earlier investigations had shown that, in culturing normal marrow in the individual's own serum and normal marrow in normal serum from another individual, the rate of erythropoiesis is about 15 per cent lower in the latter case.

Already after 9 hours, the myelopoietic cells showed signs of developing in to more mature forms, as now a few metamyelocytes and single stab-nucleated, neutrophilic granulocytes could be observed. This maturation was still more pronounced after 24 hours.

¹ Personal communication by Professor Bo C. Vahlquist.

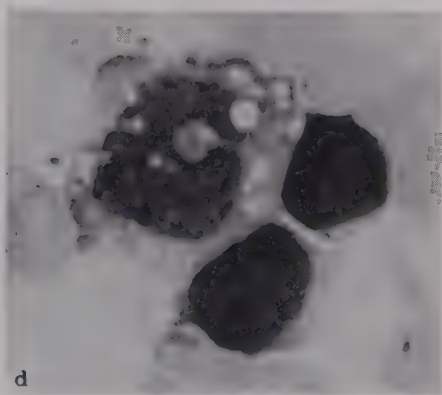
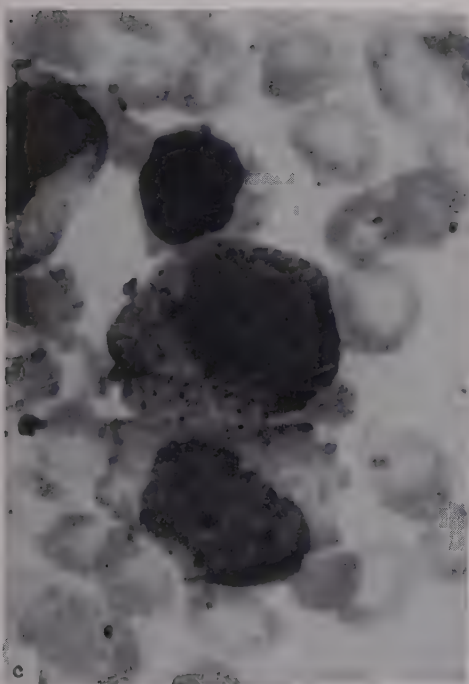
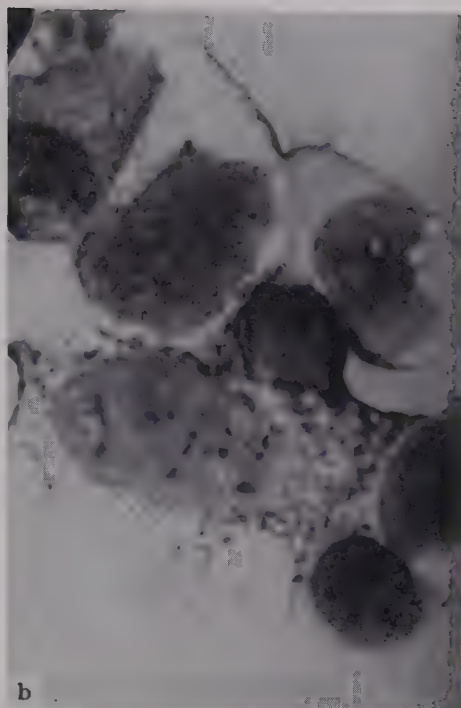
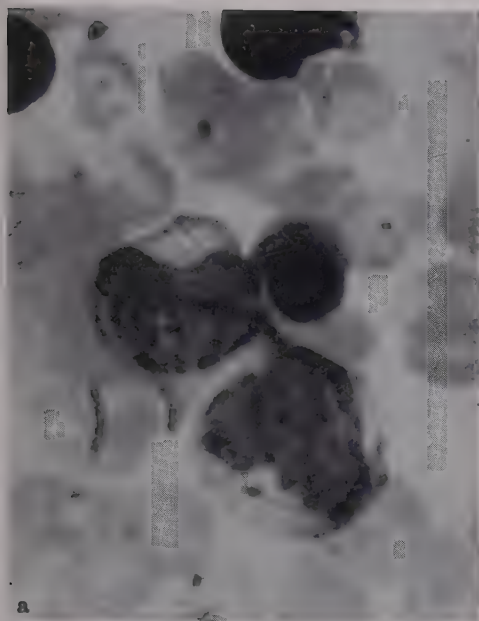


Fig. 11. Case R4. Microphotographs of bone marrow smears.

(3) Culture of normal bone marrow cells in the patient's serum: A slight decrease in the rate of erythropoiesis should be expected. However, this decrease was considerably greater than anticipated. Consequently, the patient's serum had either an inhibitory effect or lacked substances required to maintain a normal rate of erythropoiesis.

(4) Culture of the patient's bone marrow cells in his own serum with added tyrosine: This experiment gave the same result as experiment (1).

(5) Culture of the patient's bone marrow cells in his own serum with added cysteine: The cysteine was added in amounts equivalent to 0.3 mg per 100 ml. blood. In this experiment, completely normal erythropoiesis was observed, and the differentiation in the cells of myelopoiesis was more pronounced than when using the patient's own serum alone. The normalization was more pronounced than when normal serum was used.

(6) Culture of the patient's bone marrow cells in normal serum with added cysteine: The result was about the same as under (5).

Conclusion: In culturing the patient's bone marrow cells, a normalization of the cell development and maturation was observed when cysteine was added to the patient's serum, and a relative normalization when cultivation occurred in serum from a normal individual.

These in vitro experiments suggest that the patient could not fully utilize the sulfur-containing amino acids that are of importance in cell differentiation.

X-ray examination of heart and lungs, April 17, 1952. Normal findings.

X-ray examination of skeleton, April 30, 1952. Posteriorly and parietally on the right side of the skull, there was a compact condensation the size of a pea. All other skeletal structures appeared normal.

Serological tests, May 7, 1952. *Wassermann* reaction in blood negative. *Antistreptolysin* titer 22 units per ml. *Antistaphylosin* titer 28 units per ml. May 12, 1952. *Antistaphylosin* titer 5.6 units per ml.

Blood proteins, April 30, 1952. Total proteins as measured by Kjeldahl 8.5 per cent. The γ -globulin fraction was 42 per cent of total protein (fig. 12). May 17, 1952: Total proteins 8.6 per cent, thereof albumin 3.2 per cent and globulin 5.4 per cent. June 14, 1952: Total proteins 7.5 per cent. August 14, 1952: total proteins 7.5 per cent (γ -globulin 29 per cent (fig. 12)). On March 13, 1953: Total proteins 7.5 per cent.

Biopsy of bone marrow, May 9, 1952 (N. Gellerstedt, M.D.): The bone marrow was rich in cells, with moderate erythropoiesis and rather active myelopoiesis; however, the more mature stages were poorly represented. Approximately normal numbers of megakaryocytes were observed. There was no distinct proliferation of the reticular cells.

A *lymph node* excised at the same time showed chronic inflammatory reticulosis of a common type.

The findings gave no conclusive information regarding the nature of the disease.

Pathologic-Anatomic Examination (Fredrik Wahlgren, M.D.).

Within the examined parts of the *lungs*, there were wide-spread pneumonias which showed a pronounced tendency to necrosis and formation of abscesses. The necrotic masses were extremely rich in various kinds of bacteria, mostly Gram-positive cocci

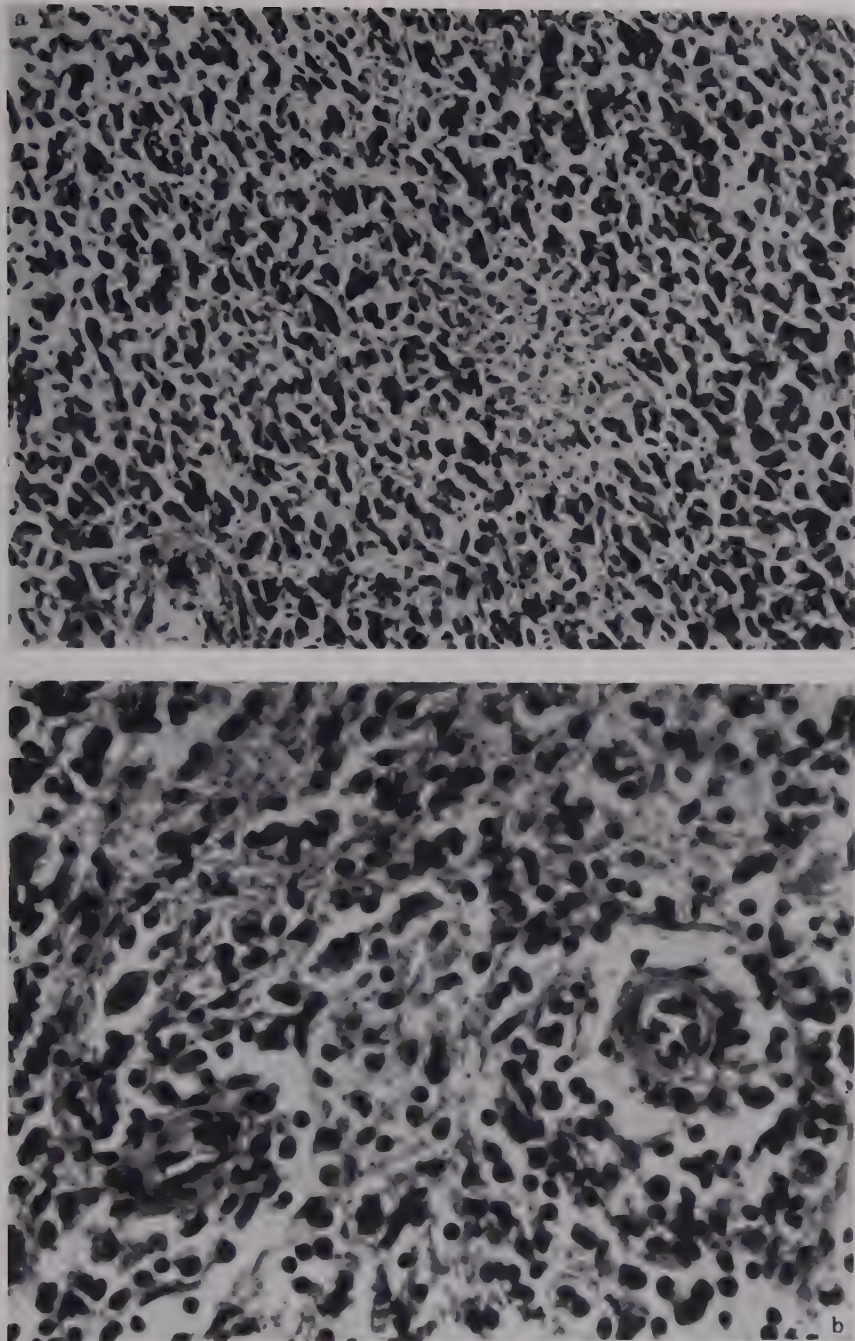


Fig. 13. Case R4. Autopsy specimens: *a*, Liver: Focus with necrotic and necrobiotic cells. Depletion of acinar structure. *b*, Spleen: Swelling of the sino-endothelial cells.

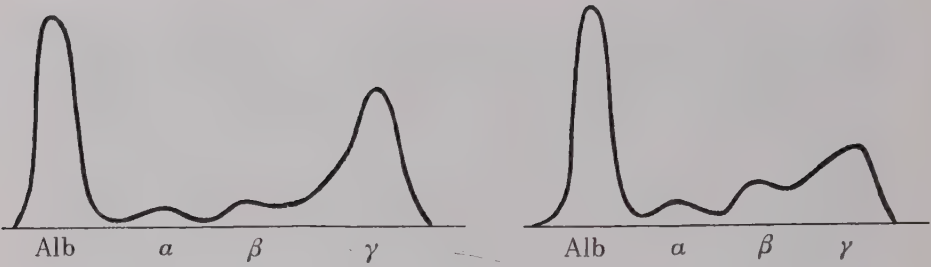


Fig. 12. Case R4. Blood proteins by electrophoresis.

30/4 1952.		14/8 1952.	
<i>Electrophoresis</i>		<i>Electrophoresis</i>	
Albumins	42 %	Total proteins	7.5 %
Alpha-globulin	5 %	Albumins	49 %
Beta-globulin	10 %	Alpha-globulin	7 %
Gamma-globulin	42 %	Beta-globulin	15 %
Albumins		Gamma-globulin	29 %
Globulins		Albumins	
$\frac{\text{Albumins}}{\text{Globulins}} = 0.72$		$\frac{\text{Albumins}}{\text{Globulins}} = 0.96$	

in large clumps. In addition, there were cocci and bacilli having a different formation. No acid-fast bacteria were seen.

The acinous architecture of the *liver* (Fig. 13a) appeared distinct. The liver cells in the central parts of the acini were largely necrotic or more or less strongly necrobiologically changed. In the periphery of the acini, the liver cells were wide and empty. The periportal connective tissue was more abundant than usual. There was no pathological concentration of cells in it. Gall passages and vessels appeared normal.

The follicles of the *spleen* (Fig. 13b) were small. They lacked germinal centers and their borders towards the pulp were rather diffuse. The pulp was devoid of blood and rich in cells. The pulp cells were not enlarged and there were no pathological cell elements in the pulp. The reticulum appeared to be somewhat increased.

The normal architecture of the *lymph nodes* was diffuse without being completely erased. The tissue contained a few enlarged reticular cells. Giant cells or eosinophilic leucocytes were not observed. The *bone marrow* was relatively poor in cells. It contained predominantly erythrocytes and their precursors. The reticulum cells appeared enlarged. There was only a small number of megakaryocytes.

Epicritic Summary

A boy, who, from the age of $2\frac{1}{2}$ months, repeatedly had furuncles and abscesses of the skin in connection with high fever. He was treated with antibiotics. No examination of the white blood cells was undertaken until he was 10 months old. He then had numerous furuncles and abscesses again, as well as high fever. The blood showed agranulocytosis and monocytosis. Bone marrow examinations showed a maturation block at the promyelocyte-

myelocyte stage. The continued course of the disease was characterized by recurring septic skin conditions and infections of the upper respiratory tract. The infections could be counteracted with antibiotics, but recurred as soon as the treatment had been discontinued for some time. Despite the infections and the pronounced granulocytopenia-agranulocytosis which persisted throughout the course of the disease, he could, with the aid of antibiotic treatment, be kept alive until the age of three years, when he died of widespread broncho-pneumonias.

The clinical course, the blood and bone marrow pictures, as well as the post mortem findings, justify the diagnosis of agranulocytosis. There were no indications that this agranulocytosis should have had an exogenous toxic or allergic origin.

CASE K2

Boy. Born June 15, 1950. Weight at birth, 3600 g. Normal delivery. Breast-fed for $2\frac{1}{2}$ months. On September 9, 1950, he was taken to an infirmary because he was pale and looked sick. He had a moderate stridor. The temperature was subfebrile, but there were no obvious signs of infection. He had a slight anemia and was treated with iron. A sternal puncture was made on September 18, 1950. The preparation showed maturation arrest in both myelopoiesis and erythropoiesis. Therefore, the child was transferred to the Children's Hospital in Boden on September 20, 1950. He was pale in spite of a hemoglobin value of 87 per cent. He had a slight stridor of a predominantly inspiratory type. There were no signs of rickets. The liver, spleen and superficial lymph nodes could not be felt. The internal organs appeared normal on physical examination.

Since the bone marrow continued to show the same type of changes as at the first examination, he was observed and treated in the hospital until November 20, 1950, when he was discharged at the request of his parents. During the entire stay at the hospital, he had a dirty-grey skin color. The paleness was in no way correlated with his hemoglobin values. On some occasions, the blood showed relative granulocytopenia. The lowest absolute number of granulocytes was 900 per cu.mm. Concerning the continued course, it is only known that the child was still alive in 1954.

Special Examinations

Blood examinations have been summarized in Table III.

Bone Marrow Examinations (N. G. Nordenson, M.D.)

September 18, 1950. Sternal punctate: Very cell-rich preparation. A slight maturation arrest in myelopoiesis with an increase in myelocytes and promyelocytes. Slight eosinophilia. Erythropoiesis was hyperplastic with strong maturation arrest; increase

showed slight maturation arrest. Increase in lymphocytes without a definite metaplasia. Reticulum very hyperplastic.

Diagnosis: Strongly reactive marrow. Chronic irritation. Systemic disease?

Tibial punctate: Very cell-rich preparation. Slight maturation arrest in myelopoiesis with an increase in myelocytes. A certain maturation block after stab nucleated cells. Erythropoiesis was slightly hyperplastic with some maturation arrest and maturation disturbance. Megakaryocytes with slight maturation arrest. Lymphocytes strongly increased; possibly metaplasia. Reticulum very hyperplastic.

Diagnosis: Strongly reactive marrow. Chronic irritation. Systemic disease? Lymphatic leukemia?

October 21, 1950. Tibial punctate: Very cell-rich preparation. Strong maturation arrest in myelopoiesis down to myeloblast-stem cells. Moderate maturation block after stab nucleated cells. Erythropoiesis was hyperplastic with maturation arrest; increase in basophilic normoblasts and proerythroblasts. Slight maturation disturbance. Megakaryocytes were normal. Strong increase in lymphocytes, but no metaplasia. Reticulum strongly hyperplastic.

Diagnosis: Reactive marrow. Systemic disease?

November 1, 1950. Tibial punctate: Cell-rich preparation. Strong maturation arrest in myelopoiesis with increase in myelocytes and promyelocytes. Maturation block after stab nucleated cells. Erythropoiesis was hyperplastic with strong maturation disturbance. Megakaryocytes were normal. Lymphocytes were strongly increased in number but showed no metaplasia. The reticulum was strongly hyperplastic.

Diagnosis: Reactive marrow. Some granulopenia. Systemic disease?

November 13, 1950. Tibial punctate: Very cell-rich preparation. Strong maturation arrest in myelopoiesis with increase in myelocytes and promyelocytes, as well as in myeloblast-stem cells. Erythropoiesis was hyperplastic with moderate maturation arrest. Megakaryocytes were normal. The reticulum was strongly hyperplastic.

Diagnosis: Reactive marrow. No specific features. Systemic disease?

X-ray examination of the skeleton, September 22 and November, 1950: No pathological changes.

Wassermann reaction in blood: negative. *Mantoux* 1 mg.: negative.

Epicritic Summary

A boy, because of paleness and relatively poor general state of health, was taken to a hospital at the age of three months. He then had a moderate congenital stridor. He was observed for a period of two months. On some occasions, he had a moderate granulocytopenia. The lowest absolute number of granyocytes was 900 per cu-mm. During the entire period, the bone marrow examinations disclosed maturation disturbances or maturation arrest in myelopoiesis, as well as in erythropoiesis. It was remarkable that, despite this disturbance in erythropoiesis, the blood gave normal values for hemoglobin and the number of erythrocytes.

Concerning the continued course, it is only known that the child was still alive in 1954.

The blood and bone marrow findings make it probable that there was a disturbance in myelopoiesis of a granulocytopenic-agranulocytotic type, in principle, similar to the one observed in the other cases described within Group I. It seems likely that this boy suffered from the same disease, but that it occurred in a less severe form.

CASE L1

Boy. Born January 31, 1950. Weight at birth 2760 g. He was breast-fed for two months, and developed normally. At the age of five months, he fell ill with fever and got several furuncles on the scalp and neck. The largest ones were of the size of hazel nuts. He was taken to an infirmary and treated there with incisions and penicillin. He became free of fever and the skin processes healed. His hemoglobin was 79 per cent. A few days after discharge, he again developed two furuncles the size of hazel nuts on the neck. These healed without treatment. Because of insufficient increase in weight and poor general state of health, he was, however, again taken to the infirmary. On October 25, 1950, a differential count of the white blood cells showed a considerable granulocytopenia with only 4.5 per cent granulocytes. The patient was transferred to the Children's Hospital in Boden on November 16, 1950. On November 26, 1950, he was sent to the Sachs' Hospital for Children in Stockholm for continued treatment and observation.

At the time of the first examination in the hospital, he had fever and signs of an infection in the respiratory tract. He soon became afebrile, and from then on the temperature was normal. During the two months of observation, his general state of health was relatively good. However, he increased poorly in weight. The weight was about 8500 g. There were no signs of rickets. He was anemic and was treated with iron. His hemoglobin rose from 46 to 75 per cent. The liver and spleen were not palpable, and there was no enlargement of the lymph nodes. With the exception of the initial granulocytopenia, the white blood cells did not exhibit any noteworthy changes. During the whole period, sternal punctates showed a reactive marrow with a maturation arrest in myelopoiesis with an increase in myelocytes and promyelocytes. Erythropoiesis showed signs of maturation disturbance, and the reticulum was strongly hyperplastic.

After discharge from the Sachs' Hospital for Children on January 13, 1951, he returned home. Concerning the continued course, it is only known that he was still alive in 1954.

Special Examinations

Blood examinations have been summarized in Table IV.

Bone Marrow Investigations (N. G. Nordenson, M.D.)

October 25, 1950. Sternal punctate: Very cell-rich preparation. There were signs of toxic myelopoiesis and strong maturation arrest down to myeloblasts. The eosinophilic cells were increased. Erythropoiesis was strongly hyperplastic with maturation arrest; increase in basophilic normoblasts. Pronounced anisocytosis and microcytosis was observed. The megakaryocytes were remarkably rare. The reticulum was strongly hyperplastic.

Diagnosis: Strongly reactive marrow. No specific characteristics.

November 17, 1950. Tibial punctate: Cell-rich preparation. There was a strong maturation arrest in myelopoiesis down to stem cells. Erythropoiesis was hyperplastic with maturation arrest; increase in basophilic normoblasts and proerythroblasts. Pronounced anisocytosis and polychromasia was observed. The megakaryocytes were normal. The lymphocytes were increased in number without metaplasia. The reticulum was strongly hyperplastic.

Diagnosis: Reactive marrow. No specific characteristics. Systemic disease?

December 20, 1950. Sternal punctate: Cell-rich preparation. There was a strong maturation arrest in myelopoiesis with an increase in myelocytes. A strong maturation disturbance was also observed in erythropoiesis, and anisocytosis was pronounced. The megakaryocytes were normal. The lymphocytes were increased without metaplasia.

Diagnosis: Reactive marrow. No specific characteristics.

January 4, 1951. Sternal punctate: Very cell-rich preparation. There was a maturation arrest in myelopoiesis with an increase of myelocytes and promyelocytes. Erythropoiesis was hyperplastic with maturation arrest, increase in basophilic normoblasts and proerythroblasts. Pronounced anisocytosis. The megakaryocytes were normal. The reticulum was strongly hyperplastic.

Diagnosis: Reactive marrow. No specific characteristics. Systemic disease?

January 10, 1951. Tibial punctate: Cell-rich preparation. There was a strong maturation arrest in myelopoiesis with an increase of myelocytes and promyelocytes. Erythropoiesis was hyperplastic with strong maturation arrest; increase in basophilic normoblasts and proerythroblasts. Pronounced anisocytosis. The megakaryocytes were normal. The lymphocytes were increased without metaplasia. The reticulum was hyperplastic.

Diagnosis: Reactive marrow. No specific characteristics.

X-ray examinations of the skeleton, November 21, 1950: No pathologic structural changes.

Mantoux 1 mg: negative.

Epicritic Summary

A boy fell ill at the age of five months with fever and furuncles on the scalp and neck. He was treated with penicillin and incisions. Shortly afterwards, he again developed a few furuncles which healed spontaneously. At

TABLE IV

Case L1. Blood examinations.

Date	Hemoglobin %	Red blood cells million per mm ³	White blood cells per mm ³	Stab cells %	Segmented neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Unspecified cells %
25/10			15,000	1	3.5	—	—	80	16.5	—
17/11	46	5.18	6,900	6.5	31.5	—	—	52	9.5	0.5
18/11	48									
20/11	47	4.76								
22/11	49	4.82								
24/11	49	4.48	6,100	1	32	0.5	1	53	10.5	2
1/12				—	44.5	2	3	44.5	6	—
2/12				—	39	3	3	47	8	—
6/12				—	36	—	—	57	7	—
9/12				0.5	17.5	0.5	2	77	2.5	—
15/12				1	31	2	1	58	7	—
20/12	46	4.30	8,300	—	23.5	1	1	72	2.5	—
22/12			10,700	—	25.5	5.5	1.5	63	4.5	—
24/12			11,900	—	35	—	1	60	4	—
26/12			6,000	1	18	2	4	70	5	—
28/12			7,900	1	37	5	—	55	2	—
29/12	64	2.90								
2/1			10,400	1	34	1	1	58	5	—
5/1			8,300	—	31	—	2	61	6	—
8/1	75	3.90	6,300	1	24	2	1	70	2	—
10/1			8,200	—	34	1	2	57	6	—
13/1			7,600	—	16.5	1	—	79.5	3	—

the age of nine months, his general state of health was poor. There were no signs of rickets, and the internal organs appeared normal. His hemoglobin was 50 per cent and the white blood cells 15,000 per cu.mm. A differential count disclosed a granulocytopenia with only 4.5 per cent granulated cells. The bone marrow was strongly affected with a significant maturation arrest in myelopoiesis. Erythropoiesis also showed maturation disturbances.

He was observed for two months. His general state of health was relatively good, but the weight increase was poor. He was treated with iron and the hemoglobin rose to 75 per cent. His liver and spleen were never palpable, and there was no enlargement of the lymph nodes. Apart from the initial granulocytopenia, the white blood cells did not show any noteworthy

changes during the period of observation. The bone marrow, on the other hand, showed significant changes the whole time with maturation arrest and maturation disturbances in myelopoiesis as well as erythropoiesis, together with a strongly hyperplastic reticulum.

Concerning the continued course, it is only known that the child was alive and healthy in 1954.

The condition of the bone marrow, and, on one occasion, the blood findings indicate a disturbance of myelopoiesis, in principle, similar to that present in granulocytopenia-agranulocytosis. Due to this, and on account of the course of the disease (carbunculosi), it seems likely that this boy suffered from the same disease as the other children reported above, although it took a milder course.

Chapter III

The Affected Children of Group II

Case A1

Boy. Born July 11, 1939. Died August 11, 1939. Cause of death: Pemphigus.

Information given by the mother: The delivery and weight at birth were normal. At the age of two weeks, he fell ill, developing red, boil-like eruptions on the body and fever. He was treated by the district physician, and died at home at the age of 31 days.

Case A3

Girl. Born September 27, 1941. Died October 26, 1941. Cause of death: Carbuncle.

Information given by the mother and the district nurse: The delivery and weight at birth were normal. The child fell ill at the age of two weeks with multiple boils and fever. She was taken to the district infirmary and was treated there for two weeks. It has not been possible to find her records in the infirmary archives. She died at the infirmary when 28 days old.

Case A5

Boy. Born January 9, 1944. Died February 10, 1944. Cause of death: Pemphigus.

Information given by the mother and the district nurse: The delivery and weight at birth were normal. He fell ill between the age of two and three weeks with fever and boils. The last few days of his life, he was treated in the district infirmary, where he died at the age of 32 days. His records could not be found in the infirmary archives.

Case C1

Boy. Born January 21, 1944. Died February 8, 1944. Cause of death: Phlegmone regionis scrotalis et perinei and sepsis.

The delivery and weight at birth were normal. He was breast-fed. At the age of 15 days he fell ill with swelling and redness of the scrotal region. The following day, he had high fever and a bluish-red discoloring of the scrotum and penis. He was taken to the surgical ward at Gällivare Hospital. The scrotum was markedly swollen and discolored and bluish-red. Tem-

perature 40.4° C. Incision was made. The boy died the following day. Autopsy was made. Apart from an edema-like infiltration of the scrotum and the abdomen, nothing abnormal had been observed. However, neither pathologic-anatomic nor blood examinations were made.

Case E1

Girl. Born February 26, 1938. Died March 26, 1938. Cause of death: "Presumably gastric disease".

Information given by the mother, the district nurse, and the district midwife: She was born two weeks before the expected time. Her weight at birth was normal, but she was slightly cyanotic shortly after delivery. She nursed well, and increased normally in weight. At the age of two weeks, she fell ill, developing small furuncles on the body, primarily on the shoulders and the neck. They increased in size. In the neck, a large bluish-red infiltration formed. She developed a cough and high fever. The district nurse saw her one day before death. Her condition was then so poor that the nurse did not think a physician could be of help. She died at the age of 30 days.

Case F2

Girl. Born February 13, 1939. Died March 19, 1939. Cause of death: „Congenital weakness“.

Information given by the mother: The delivery and weight at birth were normal. At the age of two weeks, she fell ill with boils. First, a boil developed on the neck, and, later, another one on the right anterior side of the chest. The boil on the neck became so large that the redness and swelling included the whole neck and extended some way down the back. She had fever. She died at home at the age of one month and six days. No physician or nurse saw her during her illness.

Case O3

Girl. Born July 26, 1940. Died August 29, 1940.

Information given by the mother and the district nurse: The weight at birth was 2500 g, and the delivery was normal. She was breast-fed. She was healthy the first week, but developed eruptions at the age of two weeks. These started on the abdomen as small red dots, and became gradually larger until they finally reached the size of about 1 × 1 cm. They were described as furuncles or abscesses. There was no blister formation. She had slight fever, but did not seem ill the first few days. She later became fussy, thinner, and pale. There was no cough or cold, nor was there vomiting or diarrhea. She became progressively weaker, and ate very poorly the last

few days. The urine was then a reddish color. The district physician saw the child and perscribed some ointment. The district nurse had told the physician that she thought that the abscesses looked like „Dirivaara boils“ (Dirivaara is the village where family A lives). She died at the age of one month and three days.

Case R1

Boy. Born May 9, 1941. Died June 27, 1941. Cause of death: Thrombophlebitis crur. bilat. cum septicaemia et inanitio.

The weight at birth was 2800 g. He was taken to a doctor on June 18, 1941 for blisters on the legs, and was treated with some ointment. Later, he developed diarrhea and decreased rapidly in weight. The blisters on the legs increased markedly in size, and fever developed. On June 25, 1941, he was taken to the hospital in Kalix. His general state of health was miserable. He was atrophic, dehydrated, and cyanotic. His temperature was 39°C. and the pulse was very rapid. The heart, lungs and abdomen appeared normal. On the inside of the thighs and on the groins, there were large, round sores. Between these, and extending up on the abdomen, there was a hard, red infiltration. He died on the following day. He was then one month and nineteen days old. No histological or blood examinations were made.

Chapter IV

Discussion of the Clinical and Pathological Data

The most important cases for the analysis of the clinical and pathologic-anatomic characteristics of the disease are those in Group I. Of these, cases A9, R4, K2 and L1 have been diagnosed and examined primarily by myself. Cases A9 and L1 have also been studied at the Sachs' Hospital for Children in Stockholm (H. Magnusson, M.D.), and case R4 at the Pediatric University Clinic at Uppsala (Professor B. Vahlquist). In cases D1 and T9, the diagnosis of agranulocytosis was made by physicians who did not know the genetic conditions (M. Sjöholm, M.D., and E. Sahlin, M.D., Gällivare Hospital). Pathologic-anatomic examinations of cases A9, D1, L1 and R4 have been performed by F. Wahlgren, M.D., Stockholm.

The histories of the eight cases in Group II are strikingly similar to cases A9, D1, R4 and T9 in Group I. They all belong to the same family complex as the cases in Group I. The genetic analysis has made it probable that they are all homozygotes in regard to predisposition to the same disease. Because of this, and because of the fact that malignant septic skin infections with lethal outcome are very rare in early infancy, it seems justified to conclude that the children in Group II have had the same disease and died of the same causes as those in Group I.

Two of the children in Group I, cases K2 and L1, differ from the other children in Groups I and II with respect to the clinical data and the course of their disease. In both cases, granulocytopenia could only be observed on a few single occasions. Case K2 lacked septic skin infections, a symptom which characterized all other children in Groups I and II. Both children lived at the time of termination of this investigation and were then $4\frac{1}{2}$ and 5 years old, respectively.

Case K2 was encountered by chance, and has thus not been registered according to the same principles employed in the other cases. For this reason, it has not been included in the genetic analysis.

Clinical Signs and Symptoms

All children have fallen ill at an early age. *The first symptoms of the disease in all cases except one (K2) have been due to infections. Except in case A9, these consisted primarily of septic skin infections.* In case A9, there were

first signs of an infection in the upper respiratory tract with otitis and mastoiditis. Septic infections of the skin started a month later.

The age of onset varied from 1 to 3 weeks in ten of the cases. The others fell ill at the age of $1\frac{1}{2}$, $2\frac{1}{2}$, 3, and 5 months, respectively.

The clinical signs in the initial stage, as well as during the continued course of the disease, were characterized by the appearance of septic skin infections of the type of furuncles, phlegmons, and abscesses in connection with fever. Remarkably enough, no appreciable enlargement of the lymph nodes was present. In addition, the liver and spleen were not noticeably enlarged. *Apart from the septic symptoms and the agranulocytosis, no especially characteristic symptoms of this disease have been observed. All these symptoms initially, as well as during the continued course, can be explained as a consequence of the agranulocytosis and the strongly decreased resistance to infections.*

The most important observation in the peripheral blood was agranulocytosis or grave granulocytopenia which was present in cases A9, D1, R4 and T9.

Case A9, which was observed for over three months, had more than 1000 granulocytes per cu.mm. blood on only one occasion during this period. During the rest of the time, the number of granulocytes varied between 40 and 500 per cu.mm.

Case R4, which was observed for over 2 years, had only on one occasion more than 1000 granulocytes per cu.mm. blood. During the rest of the time, the number of granulocytes varied between 40 and 500 per cu.mm.

Cases D1 and T9 were only observed for a few days before death, and they both had a high degree of granulocytopenia.

Cases K2 and L1 had granulocytopenia only on a few single occasions.

A remarkable finding was the high number of monocytes observed in cases A9 and R4. In both cases, the monocytes, as a rule, made up 50 per cent of the total number of white blood cells. Monocytosis was also observed in case T9. In the two differential counts that could be made, the monocytes made up 16 per cent and 20 per cent, respectively, of the total number of leucocytes.

Agranulocytosis with concurrent monocytosis is not common, but has been described both in adults and children (Thaddae and Bakalos 1939, Tobler and Buser-Pluss 1942, Stodtmeister 1942). Tobler and Buser-Pluss could demonstrate experimentally that the monocytes of their patient had a considerably stronger phagocytic function than those of controls. They believe that this increase in phagocytic function compensated for the loss of granulocytes.

It is possible that in cases A9, R4 and T9 the monocytosis was the result of such a compensatory mechanism. The antibiotic treatment of the infec-

tions in cases A9 and R4 must have been the main cause of the prolongation of life, but, even so, it is likely that an increased resistance towards infections developed in these cases and influenced the course of the disease.

In contrast to the grave changes in the leucocytes, the affected children displayed only moderate and uncharacteristic changes in the red blood cells.

The first observed hemoglobin value in case A9 was 75 per cent. During the first two weeks, this child received blood transfusions three times to improve its general condition. During the following three months, the child did not receive any transfusions. The hemoglobin decreased slowly from 100 to 75 per cent during this period. The number of erythrocytes decreased from 4.7 to 2.8 million per cu.mm. The color index remained close to unity.

Case R4, who had suffered from repeated septic infections for seven months before any blood examination was made, had then 53 per cent hemoglobin and 3.5 million erythrocytes per cu.mm. During the continued course (over two years), the hemoglobin values fell between 51 and 64 per cent, and the number of erythrocytes between 3.1 and 4.0 million per cu.mm.

Case T9, two days before death, had a hemoglobin of 97 per cent and 5.0 million red blood cells per cu.mm. Case D1 had a hemoglobin of 44 per cent and 2.3 million erythrocytes per cu.mm. five days before death.

During the period of observation, case K2 had hemoglobin values between 80 and 95 per cent and a number of erythrocytes between 3.8 and 4.3 million per cu.mm.

Case L1 had hemoglobin values of 46 to 49 per cent with 4.3 to 5.1 million erythrocytes per cu.mm. during the first month of observation. During the following months, the hemoglobin rose to 75 per cent.

A disease of this type characterized by repeated septic infections with fever, must put great demands on erythropoiesis and this should lead to some anemia. The changes in the erythrocytes observed in these children may well be assumed to have developed in this manner, and *the anemias of a moderate degree that have been observed should be regarded as secondary anemias.*

Normal thrombocyte values were observed in cases A9, R4, K2 and L1. Case D1 had a high number of thrombocytes, i.e. 590,000 per cu.mm.

The bone marrow was studied repeatedly during the whole period of observation in cases A9, R4, K2 and L1. In cases A9 and R4, *the essential observation was a pronounced maturation arrest in myelopoiesis. The myeloid cell series was completely dominated by myelocytes and promyelocytes. These cell types often appeared in atypical forms with lobed and vacuolated nuclei.* There was an almost complete maturation arrest at the myelocyte state. There was also a considerable hyperplasia of the reticular cells, particularly the lymphoid and monocytoid forms.

In case A9, erythropoiesis was largely intact up to the last few weeks, when signs of maturation arrest and maturation disturbance appeared. In case K4, erythropoiesis showed signs of poor activity the whole time.

In case R4, a number of interesting studies and observations could be made in *culture experiments with the patient's bone marrow*. The results of these *in vitro* experiments suggest that the patient's serum was deficient in such substances as are required for the normal maturation of the bone marrow cells. This was particularly true for myelopoiesis but also for erythropoiesis. An increased maturation of the cells was obtained upon the addition of cysteine to the medium. *Judging from these experiments, it may be assumed that the patient had a deficient ability to utilize the sulphur-containing amino acids which are of importance in cell differentiation*. Because of this, *therapeutic experiments were performed in which cysteine was given to the patient orally and intravenously*. No conspicuous improvement could, however, be observed.

Cases K2 and L1 both showed changes in the bone marrow of, in principle, the same kind. There was found a considerable maturation arrest in myelopoiesis with an increase in myelocytes and promyelocytes and a relative decrease in the more mature forms. However, in none of these cases was the maturation arrest on any occasion as pronounced as in cases A9 and R4. Erythropoiesis was pathological in both cases with maturation arrest and maturation disturbances.

A remarkable finding in cases K2 and L1 was the discrepancy between the bone marrow changes on the one hand and the peripheral blood picture on the other. On account of the changes in the bone marrow, it is surprising that, as a rule, no granulocytopenia was present in the blood.

Of the 14 cases in Groups I and II, 13 have had severe septic infections of the skin manifested as furuncles, phlegmons, abscesses and necrotic sores. Infections of the upper respiratory tract, otitis, mastoiditis and bronchial pneumonia have also been found. *In the 12 cases that died, the infections have been the immediate cause of death*.

In cases A9 and R4, pathogenic strains of *Staphylococcus aureus* have repeatedly been found in cultures from the skin processes. In addition, growth of α -streptococcus, *Bacillus pyocyaneus* and coliform bacteria has been obtained in a few samples.

The dominating role of the septic infections is not surprising. In all known agranulocytotic conditions, such infections are common as the immunobiologic protective mechanisms are poorly developed in infants. *However, in case R4 the immunobiologic protective mechanisms in respect to serologic factors were fairly good (antistaphylosin titer 28 units per ml. Gamma globulin 42*

per cent of total serum protein!)). This suggests that the infection susceptibility was mostly of cellular origin.

In agranulocytotic conditions in infants described in the literature, suppurative skin afflictions have often been part of the clinical picture.

An important part of the data (Group II) have been collected as histories. The very presence of severe septic skin infections has been considered diagnostic. Thus, no calculations can be made of the frequency of skin infections. There are, however, grounds for assuming it to be very high. It is, however, possible that a number of non-identified cases of the disease could have died from other types of infections before pyogenic skin infections had time to develop.

Therapy

The only therapy available at present is treatment of the infections with antibiotics. Cases K9 and R4 had probably not lived as long as they did without this therapy. It appears probable that the importance of the antibiotic treatment was not limited to counteracting the infections. If the child's life is prolonged with this therapy, conditions are also established allowing the immunobiologic protective mechanisms against infections to develop.

It is possible that the observations made in case R4 on the in vitro maturation conditions of the bone marrow cells, and particularly the role of cysteine, may form the basis of a more causal therapy in the future. This type of therapy would involve an intervention in the maturation of the bone marrow cells, which process would thus be corrected by substitution in one way or another.

Pathology

In all cases, a relatively cell-poor bone marrow was observed containing mainly erythrocyte precursors. The cells of the myeloid system were very poorly represented. *The changes in spleen, liver and lymph nodes were similar, in principle, in all cases.* In the spleen, the follicles were small and lacked germinal centers. *The reticulum was increased (cases R4 and T9) and the sinus endothelial cells enlarged (cases A9 and D1).* In the liver, there was fattification of the cells (A9, D1, T9) and enlargement of the Kupffer astral cells (A9, D1, T9). No phagocytosis was observed, but the Kupffer astral cells contained iron pigment in cases A9 and T9. In the lymph nodes, an enlargement of the sinus endothelium was observed in cases A9 and T9, and an increase in the reticulum in cases R4, T9, and A9 (in biopsy material). An enlargement in the alveolar septae of the lungs was observed in case A9. They were wide and rich in lymphocytoid cells. The reticular cells were enlarged.

It should be pointed out that *no signs of tumor disease, myelogenous or lymphatic leukemia, or aleukemic lymphadenosis could be demonstrated. The signs of an involvement of the reticuloendothelial system which were observed in all cases can hardly be considered as indications of a systemic disease of a reticulosis type. It appears probable that these changes were secondary and an expression of the state of irritation caused by the infection*¹. Perhaps the lack of leucocytic defense has contributed to these changes.

¹ When this study was almost completed, an interesting discussion of this subject was published by Nordenson (1955).

Chapter V

Different Types of Agranulocytosis

Hematologically, agranulocytosis is characterized by an isolated disappearance of granulocytes from the blood with coincident intact erythropoiesis and thrombopoiesis. Signs and symptoms vary considerably. Angina, necrosis, septic skin processes and icterus may or may not be present. The only constant characteristics of the disease are the changes in the blood and bone marrow.

Agranulocytotic conditions are usually divided into the following types:

1. A form with acute-peracute onset with high fever of septic type, necrotic angina, and strongly affected general state. The disease either quickly leads to death, or else the patient recovers in a relatively short time. This form was first described by Schultz (1922).

2. More slowly developing forms which either lead to death after a relatively long time or slowly turn to complete recovery. These forms are often combined with disturbances in erythropoiesis and thrombopoiesis. The toxic infectious forms belong to this group. They lead over to aleukemia and pancytopenia.

3. So-called "Begleitagranulozytosen" where the disappearance of granulocytes is a consequence of a bone marrow disease which destroys, among other things, the myelogenous system (leucosis, tumors, reticulosis).

Etiology

The acute-peracute form of agranulocytosis (the so-called Schultz type) is caused by substances containing a benzene ring. This form has long been considered the result of a violent allergic reaction, an anaphylactic shock towards such substances. The most common allergen is amidopyrine. Other substances such as salvarsan, bismuth, dinitrophenol, gold compounds and sulfonamides, can also play an etiologic role. Their effect is slower than that of amidopyrine and, as a rule, they give rise to the toxic type of agranulocytosis. As a cause of the latter form, infections of an acute or chronic nature have been discussed in the literature.

Pathogenesis

The pathogenesis of the agranulocytotic conditions is incompletely known. Theoretically, agranulocytotic conditions can arise from the following causes:

1. Destruction of the myelogenic tissue.
2. Maturation arrest in myelopoiesis.
3. Disturbances affecting the outflow of the neutrophilic leucocytes from the bone marrow.
4. A peripheral, abnormally high destruction of leucocytes, so-called leucolysis. Such a leucolysis has been difficult to establish, but without assuming its presence, it is, for example, not possible to explain the peracute amidopyrine agranulocytosis. In such cases, it has been shown that the granulocytes can disappear from the peripheral blood in a few hours (Moeschlin 1952).

Among the possibilities just mentioned, maturation arrest has long been considered an essential cause in the origin of agranulocytosis. This maturation arrest can be studied in bone marrow samples. The observations of the morphology of the different cell elements and their relative frequencies form an important basis for the diagnosis of these conditions. They also are very important for following the course of the disease and for determining the prognosis. Two main types of bone marrow changes can be observed:

1. The "classical picture" with a complete depletion of myelopoiesis. Of the cells of the myelogenic series, only a few myelocytes and promyelocytes are present. These often display pathologic forms. At the same time, there is often a considerable increase in the reticular cells, particularly the lymphoid forms and the macrophages, especially the plasmocytous forms.
2. A bone marrow characterized by an enormous cell hyperplasia which especially affects the promyelocytes. More mature granulocytes, on the other hand, are absent or occur in very small numbers only. These changes can most closely be described as a maturation arrest. Fitz-Hugh and Krumbhar (1932) have stressed that this type of bone marrow can be found also in cases with very severe and fatal agranulocytosis.

Between these two main types of bone marrow patterns, there are intermediate forms.

The question of a peripheral destruction of granulocytes as a cause of granulocytopenia and agranulocytosis has received much attention during the last few years. Numerous investigators have demonstrated the existence of specific substances in the blood serum which destroy different cell elements. Thus, Ackroyd (1949) demonstrated a thrombocyte agglutinin in patients with Sedormid purpura. Harrington and co-workers showed, in 1951, that eight individuals with idiopathic thrombocytopenic purpura had a thrombocytopenia-causing substance in the blood associated with the globulin fraction. Evans (1951) demonstrated a thrombocyte agglutinin in

blood of patients with idiopathic thrombocytopenic purpura. He recognized the similarity with hemolytic anemia (cf. Coombs' test) and suggested the name "immuno-thrombocytopenia" for certain cases of thrombocytopenic purpura.

In 1952, Moeschlin and Wagner were able to show that the blood of individuals with amidopyrine agranulocytosis contained a granulocytopenic factor. They could establish that an agglutinin was involved. In later studies (1954), Moeschlin and co-workers have been able to verify their observations further, and show that the agglutinated granulocytes, which disappear from the peripheral blood after agglutination, adhere in the form of leucocyte emboli in the lung capillaries, where they are destroyed.

Moeschlin believes that the different types of bone marrow changes which can arise in agranulocytosis depend on the degree and duration of the peripheral leucocyte destruction and are an expression for the thusly-arisen different degrees of functional stress of the bone marrow. In a first acute stage, the marrow reacts with an attempt to maximum production, resulting in a hyperplasia with a maturation arrest. In later stages, the activity decreases, resulting in a more or less cell-poor bone marrow.

This explanation of the origin of the different bone marrow changes seems reasonable in many cases.

Moeschlin has introduced the concept "*immuno-agranulocytosis*" and believes that, for example, in amidopyrin agranulocytosis, an immunization against amidopyrin has arisen. He also thinks that a number of other agranulocytic and granulocytopenic conditions are due to immunization processes with the formation of antibodies in the form of agglutinins. He divides the agranulocytic conditions according to their pathogeneses as follows:

1. Immuno-agranulocytosis and immuno-leukopenia

- a) Leucocyte agglutinins due to drug sensitization: amidopyrin, butozolidin, hydantoin, sulfonamides.
- b) Leucocyte agglutinins of other immunological origin: primary atypical pneumonia, infectious mononucleosis, virus granulocytosis of cats, cyclic agranulocytosis, transitory granulocytopenia of the newborn, Felty syndrome, lupus erythematoses and other chronic immunogranulocytopenias.
- c) Leucocyte agglutinins of unknown origin.

2. Primary bone marrow damage (usually with pancytopenia)

- a) Due to radiation: X-ray, radium etc.
- b) Toxic: benzol, chloromycetin, urethan, gold etc.

3. Bone marrow obliteration (usually with compensatory extramedullary myelopoiesis):

- a) Osteosclerosis
- b) Bone marrow infection (Rohr)
- c) Neoplastic infiltration:
carcinomatosis, sarcomatosis, myeloma, leukemia.

Chapter VI

Agranulocytosis in Infancy and Childhood

The acute or peracute form of agranulocytosis (of the type described by Schultz) affects, above all, individuals over middle age. Only few cases have been described in children. The most common type of agranulocytosis in childhood is the so-called "Begleitagranulozytose" connected with leukemic conditions. The second most common agranulocytotic conditions belong to the toxic-infectious group. There are relatively few publications on agranulocytosis in children, most being single case reports. Apart from cases of "Begleitagranulozytose", these publications concern benign agranulocytoses caused by different chemicals, most of which belong to currently-used drugs, the prime example being the sulfa group. However, considering the widespread use of these drugs, especially in pediatrics, the frequency of such harmful effects is considerably low. Incidents of toxic agranulocytosis have also been reported where these offending drugs have not been used, or where there have not been signs of other toxic influences. In such cases, the agranulocytosis has been assumed to be caused by the prevailing infection. The agranulocytotic states have been of a relatively short duration, and the children have, as a rule, recovered when the drugs were discontinued, or when the infection was alleviated.

Pronounced granulocytopenia and agranulocytosis of long duration have also been described in children. The concept of *chronic granulocytopenia in children* was coined by Fanconi (1941). Nordmark (1939), Hotz (1941), Salomonsen (1948), Ström (1949), Tobler (1942), Vahlquist and Anjou (1952) and von Vrtilek (1952) have described such conditions.

With regard to the age of onset, the signs and symptoms and the course of disease, two of these cases are of special interest here.

The first case was described by Hotz in 1941, and concerned a twin girl who fell ill at the age of two weeks with fever and furuncles on the legs and abdomen. For a long time she was treated as an outpatient for her recurring septic skin condition. She was taken to a hospital when four months old, and it was found that she had agranulocytosis. An examination of the peripheral blood gave the following information: hemoglobin 57 per cent, erythrocytes 3.82 million per cu.mm., leucocytes 5320 per cu.mm. Differential count: metamyelocytes 2 per cent, stab cells 3 per cent, segmented neutrophils 1 per cent, eosinophils 1 per cent, plasma cells 2 per

cent, lymphocytes 84 per cent and monocytes 7 per cent. Her spleen and superficial lymph nodes could not be felt. Her liver was increased and reached 2 cm below the navel. A bacteriological culture from her abscesses yielded growth of staphylococci.

The bone marrow was markedly hyperplastic. The number of erythroblasts was increased. The most severe disturbance was observed in leucopoiesis, in which immature monocytes and promyelocytes dominated. Transition forms to myeloblasts were also found. *Particularly striking were giant forms of myeloblasts with vacuoles and occasional lobulated nuclei.* The bone marrow displayed signs of a pronounced maturation arrest of myelopoiesis commonly found in agranulocytosis. Consequently, leukemia could be excluded. It was not possible to decide what was the cause of this agranulocytosis, and it did not seem reasonable to assume that furunculosis alone could have given rise to this condition.

Apart from the local treatment of the furuncles and abscesses, prontosil and injections of nucleotrat were given. After this, a temporary increase of the neutrophils was observed. Otherwise, a more or less pronounced agranulocytosis prevailed during the entire period of observation consisting of somewhat more than four months. During this time, the child had pneumonia, as well as recurring furunculosis. When discharged from the hospital, she had 6350 leucocytes per cu.mm., of which only 6 per cent were granulated cells. At a control 2½ years later, she was healthy and her blood was completely normal. She had had no further furuncles. An examination of her twin brother revealed normal blood findings. The microphotographs of the bone marrow of this girl published by Hotz (1941) are very similar to the corresponding microphotographs of my cases A9 and R4. The similarity is particularly striking for the pathological promyelocytes and myelocytes.

The second case was described by Tobler in 1942. This was a girl who, at the age of three weeks, got paronychias and, later, recurring furuncles on the back of her head. At the age of four months, she had an infection with fever, probably influenza, followed by a bilateral otitis media. Her disease was further complicated by furuncles of the neck with extensive skin infiltration. In spite of all these infections, she developed reasonably well. At the age of nine months, she had a lacunar angina with high fever, followed by bronchial pneumonia three weeks later. She was treated with Cibazol for four days, and recovered. At the age of ten months, she again got high fever and angina with considerable swelling of the submaxillary lymph nodes. A few days later, she had a new otitis media, and recovered also this time with Cibazol treatment. An examination of her blood showed agranulocytosis. She had 7250 leucocytes with 3 per cent stab cells, 2 per cent segmented cells, 2 per cent eosinophils, 1 per cent basophils, 67 per

cent lymphocytes, 22 per cent monocytes and 3 per cent plasma cells. The red blood cells were normal and the thrombocytes 940,000 per cu.mm.

The bone marrow displayed a maturation arrest with an increase of immature myeloid cells which was especially pronounced for myelocytes and metamyelocytes. *Marked toxic disturbances, vacuolized nuclei, and atypical forms of nuclei were observed.* She received blood transfusions and pentose nucleotides. After the dissection of a vein for the blood transfusion, she got a wound streptococcal infection with a rash as in scarlet fever.

During the whole course of her disease, she had a more or less pronounced granulocytopenia or agranulocytosis. The monocytes were between 11 and 33 per cent of the total number of leucocytes. She was treated in the hospital for somewhat more than six months. When discharged, she had 8500 leucocytes per cu.mm., of which 2.5 per cent were stab cells, 6 per cent segmented cells (726 granulocytes per cu.mm.) and 13.5 per cent monocytes.

These two cases are, in many respects, similar to those presented in this report. Both of them had recurring septic skin conditions and other infections since infancy. Both displayed agranulocytosis. No specific toxic causes were disclosed. It is true that both of them received sulfa medication to counteract the infections, but, as far as can be judged, with good therapeutic effect. In the case described by Hotz, the agranulocytosis was observed already before these drugs were used and prevailed for four months. Nevertheless, the blood was normal at the control 2½ years later. Both cases were characterized by a prolonged course. In the case described by Tobler in which the agranulocytosis was observed for six months, the continued course is unknown. Both cases displayed a maturation arrest of the bone marrow, which contained numerous pathological types of myelocytes and myeloblasts with vacuolated and lobulated nuclei. By and large, erythropoiesis was normal. One of the cases had a pronounced monocytosis. Concerning the question if these two cases were genetically caused, no answer can be given. They may or may not have been induced by gene difference. The case of Hotz was a twin: but the other one displayed a normal blood pattern. However, they were non-identical twins.

Cyclic Neutropenia

Cyclic neutropenia is a special type of granulocytopenia and agranulocytosis. It was first described by Hale in 1910 and has been studied in detail by Vahlquist (1946) and Reinmann (1949), and it is now recognized as a specific clinical entity.

This disease is characterized by a pronounced neutropenia or agranulocytosis occurring with an interval of about 21 days. The neutropenic phase has a duration of five to eight days, and is usually followed by moderate

fever and local mucous changes of an apthous type in the mouth and throat. Occasionally the infections may be so severe and the agranulocytosis so pronounced that the disease resembles the agranulocytosis of Schultz's type. As soon as the blood becomes normal, the patient recovers promptly. Such periods with neutropenia may recur for several years without undue influence on the general condition of health.

The cause of this disease is unknown. It has been assumed to be congenital. Some twenty cases have been described in literature. In about half of them, the disease was first observed in childhood. It is possible that genetic factors are of importance, but no specific genetic studies have been made. The pathogenesis is also questionable. Moeschlin (1954) believes that this disease belongs to the group of immuno-leukopenias

Transitory Granulocytopenia of the Newborn

Slobodi in 1950 and Lehndorff in 1951 described two siblings with pronounced neonatal granulocytopenia. This granulocytopenia had a duration of 2-3 weeks, after which the blood quickly returned to normal. During the disease, the bone marrow displayed a pronounced maturation arrest of the promyelocyte stage. Lehndorff believes that the granulocytopenia was a toxic allergic phenomenon "and is to be considered as a manifestation of the allergy of the newborn". Moeschlin (1954) believes that this condition belongs to the group of immuno-leukopenias.

Chapter VII

Genetic Factors in Agranulocytosis and Granulocytopenia

In the available literature, I have not been able to find any reports of agranulocytosis with a definite genetic etiology. Several cases with leukopenia in the same family have been reported by Gänsslen (1941), Manzini and Parvis (1947), Hattersley (1947) and Bousser and Nedey (1948).

Gänsslen (1941) reported a pedigree of neutropenia with seven cases distributed over three generations. He claimed this to be a case of dominant inheritance. He did not observe any maturation arrest in the bone marrow. Huber (1939) claimed that certain cases of panmyelophthisis should be due to dominant genetic factors. Several cases of eosinophilia in the same family have been reported, among others, by Malmberg (1939). With the exception of these conditions for which the genetic etiology can be accepted only as a suggestion, the only clear-cut genetic condition of the leukocytes is the so-called Pelger nuclear anomaly (cf. Nachtsheim 1941).

Chapter VIII

Genetic Aspects of Infantile Agranulocytosis

by

JAN A. BÖÖK and ROLF KOSTMANN

Clinical Definition

Infantile agranulocytosis is first described in 1950 by Kostmann who interpreted the disease as a hereditary reticulosis with a monohybrid recessive inheritance. It is characterized by a more or less complete block in the maturation of the granulocytes. As a consequence, the peripheral blood displays agranulocytosis or a varying degree of granulocytopenia. Resistance against infection is low and affected individuals finally succumb to it, even if life can be somewhat prolonged with antibiotic therapy. Staphylococci infections are most common. Characteristic clinical signs are cutaneous boils and septicemia. Without treatment, the affected infants die within a few months of birth.

Nosography and Demography

The disease was discovered in a North-Swedish geographical isolate. The location of this region is shown in Fig. 1 (page 8). The area is 1073 sq. miles and, in 1950, the population totalled 9215. It is a typical farming and lumber district and, since 1644, has been a separate parish, Överkalix. Apart from the geographical location, the relative isolation is due to scarcity of roads and lack of railways.

The demographic features of this region have been similar to those of the isolates investigated by Sjögren (1932 and 1935) and Böök (1953). From 1900 to 1950, the population increased from 5539 to 9215 (cf. Table V).

The crude birth rates, although decreasing during the last 10 years, have been considerably higher than the average for Sweden (cf. Table VI).

Up to 1940, the general mobility of the population, as measured by crude migration rates, was relatively low (cf. Fig. 14, p. 66).

In conclusion, it can be stated that the demographic and geographic characteristics of the investigated area justify its classification as an isolate in terms of population genetics.

TABLE V

Parish of Överkalix. Population 1900-50.

Year	Total population		
	Males	Females	Total
1900	2,862	2,671	5,539
10	3,135	2,878	6,013
20	3,433	3,084	6,517
30	3,835	3,561	7,396
40	4,463	4,101	8,564
50	4,800	4,415	9,215

TABLE VI

Crude birth rates per 1,000 population.

Year	Parish of Överkalix	County of Norrbotten	Sweden
1931/35	29.2	22.9	14.1
1936/40	28.7	22.7	14.8
1941/45	31.1	25.7	18.7
1946/50	25.7	23.6	18.2

It should, of course, be observed that "isolate" is a *relative* concept referring to a group of people for whom matings within the group are significantly commoner than matings outside the group. Full discussions have been given by Wahlund (1928) and Dahlberg (1938 and 1947).

Collection of Data

The original family found by Kostmann was family A of the major pedigree (cf. Fig. 15, p. 67). The parents were third cousins. Starting from the common ancestors of these parents, all descendants were registered. A preliminary pedigree was drawn up which contained all sibships in which at least one child had died of unknown cause during infancy.

From the observations on family A which contained the cases A1, A3, A5 and A9, it was decided what signs and symptoms the registration of further *propositi* had to be based on.

The following Definition of a Propositus was agreed upon: *a child who had died during infancy displaying cutaneous boils and septicemia with or without confirming blood and bone marrow pathology, or still living and displaying the same symptoms and confirming pathology.*

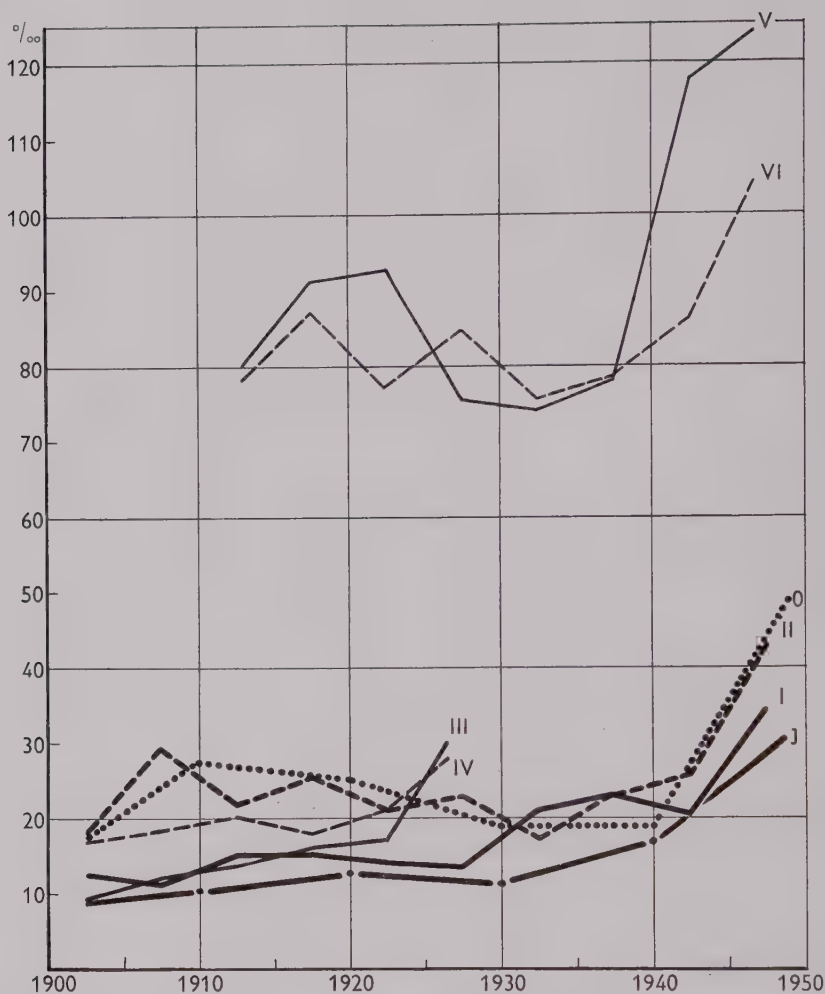
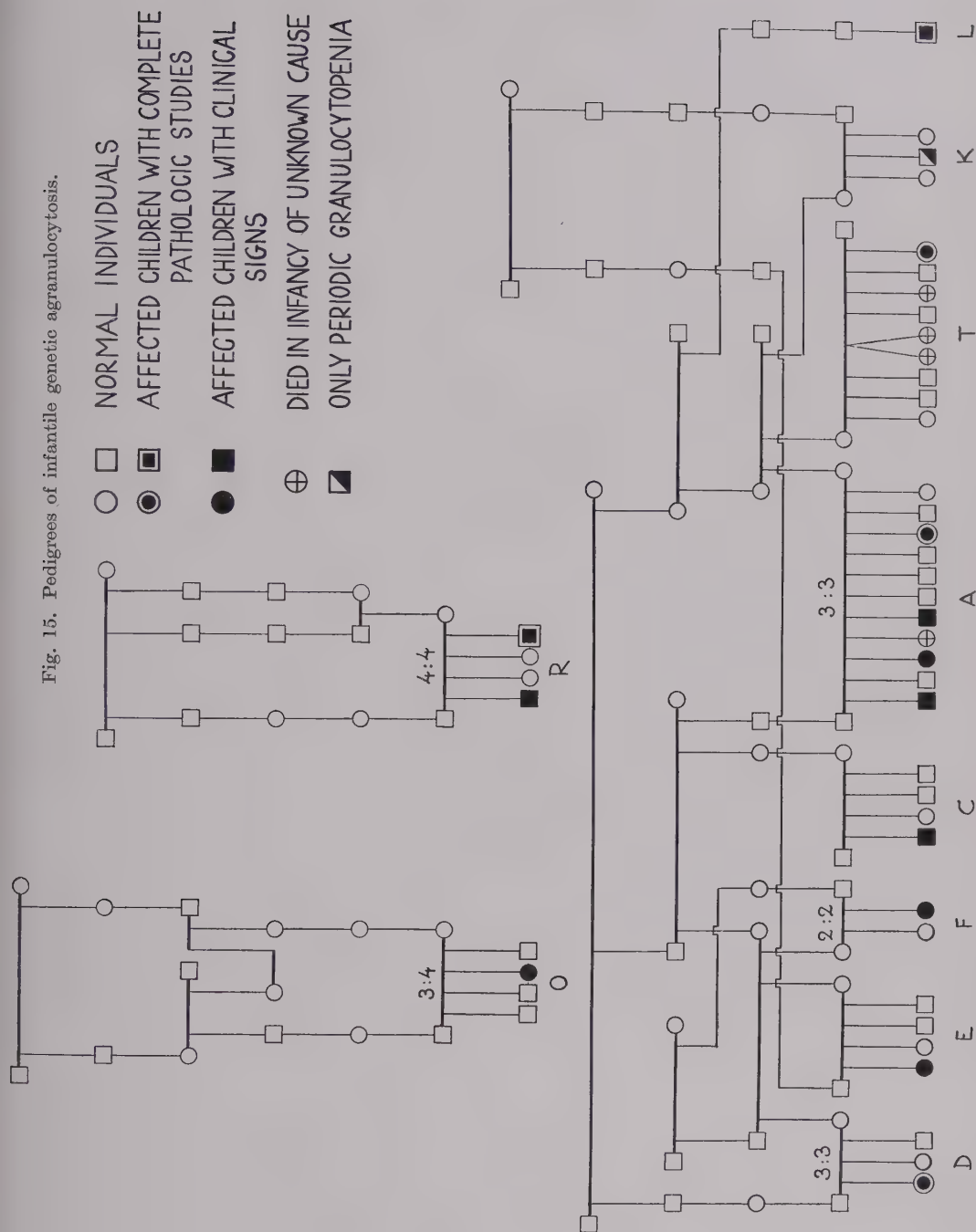


Fig. 14. Crude migration rates per 1000. I and II, in- and outmigrations, respectively, for the North-Swedish isolates investigated by Böök (1953). III and IV, in- and outmigrations, respectively, for the North-Swedish isolates investigated by Sjögren (1932 and 1935). V and VI, in- and outmigrations, respectively, for the cities of Norrbotten county. J and O, in- and outmigrations, respectively, for the parish of Överkalix.

As sufficient information could be obtained only for the last generation, the revised pedigree became considerably smaller. At this stage, it contained the families A, D, E, F and L with a total of 8 affected children. The first of these children was born in 1931.

It was then decided to make a complete registration of all children with infantile agranulocytosis born from 1931 to 1953, inclusively. For this

Fig. 15. Pedigrees of infantile genetic agranulocytosis.



purpose, the death certificates for all children who had died before the age of one year were scrutinized for this period. For every case where the cause of death was in doubt, further information was obtained by interviewing the parents, the local physician and the district nurses. This screening procedure gave only one new addition, O3, to the already-known cases.

While this work was in progress, three additional cases were reported by physicians, two from the parish of Gällivare and one from the parish of Nederkalix, all in the same county but outside the investigation area. The first two of these cases, C1 and T9, were shown to belong to the major pedigree. In both families, the mothers were born in the investigation area and the fathers had ancestors there. Family R came from Nederkalix, which is a neighbouring parish.

Consequently, all the cases so far known have been derived from a fairly circumscribed geographical area.

With reference to the definition of *propositi* given above, we are satisfied that the registration within the investigation area during 1931–1953 was complete. All genealogic data have been obtained through parish registers.

Genetic Analysis of Family Data

We are concerned with a lethal condition which showed accumulation in one major pedigree, and which was repeated in two sibships. Therefore, to begin with, the hypothesis of a single recessive gene difference should be tested.

In the pedigrees (Fig. 15), family K has been excluded from the genetic analysis because the child K2 was not ascertained to be a *propositus* according to definition. This child displayed only a moderate granulocytopenia on a few occasions and had no cutaneous infections or septicemia. It is of course possible that this case as well as case L1 is a heterozygote. This left nine families with a total of thirteen affected children. In all families, the parents and siblings have been subjected to blood studies. Bone marrow studies were made in four parents. However pathologic changes were found in none of them. There is thus so far a clear-cut difference between affected and nonaffected.

Consanguinity

In five of the nine families, the parents were related, in one as 2:2, in two as 3:3, in one as 3:4 and in one as 4:4. No data on consanguinity rates are available for this particular region. However, there is no reason to assume that the mean coefficient of inbreeding (α) should differ appreciably from another region of similar type in the same county investigated by Böök

(1956), who estimated $\alpha \geq 0.002$. This was calculated on consanguinities 1:2, 2:2, 2:3, 3:3, 3:4, 4:4 4:5 and 5:5. If we calculate the mean inbreeding coefficient (α) for the nine families according to the equation where r_i is

$$\alpha = \frac{\sum_i (r_i n_i)}{\sum_i (n_i)}$$

the degree of relationship and n_i the number in the i -th class, we obtain $\alpha = 0.012$. This should be about 6 times higher than expected for random families.

Furthermore, the fact that seven of the families could be referred to a common ancestral pair strongly indicates a recessive type of inheritance.

Test of simple recessive inheritance

The data have been obtained partly as a complete ascertainment of all cases within the population of the investigation area during 1931–1953, and partly through affected individuals born outside this area but genetically derived from it. All affected children except R1 are *propositi*.

In Table VII (p. 70), a test of simple recessive inheritance has been made according to the *a priori method*, devised by Apert and Bernstein (cf. Bernstein 1929 and Macklin 1938). The *observed and expected figures are in good agreement*. Strictly, however, it is not quite correct to include the families C, L and R, as they do not belong to the complete ascertainment. On the other hand, as shown in table VIII (p. 70, the exclusion of these families is immaterial. We obtain a difference of 0.24 between observed and expected affected children, which is less than one standard deviation (1.74).

In the major pedigree, there are four children, including one pair of twins of unknown zygosity, who died in infancy. Although the occurrence of signs typical of infantile agranulocytosis in these children was denied, there is always the possibility that such signs were overlooked. However, the evidence of a simple recessive inheritance as presented here seems sufficient to make superfluous the tentative inclusion of all or some of these cases in the calculations.

In summary, the genetic analysis of the family data has lead to the conclusion that infantile agranulocytosis, as observed in this North-Swedish population, is basically due to a single recessive gene difference. Consequently, we suggest that this new disease entity should be called INFANTILE GENETIC AGRANULOCYTOSIS.

Population Genetics

The detection of this new recessive lethal in man represents another example of the accumulation of a mutation in a particular isolate. New recessive mutations arise continuously, but their chances of survival depend

TABLE VII

Infantile genetic agranulocytosis. Test of simple recessive inheritance. *A priori method*. As affected have been included all individuals with complete pathologic studies and those with typical clinical manifestations of cutaneous infections.

Sibship size	Total no. of sibs	No. of affected children		No. of normal children		χ^2 $\Sigma (o-c)^2/c$
		<i>o</i>	<i>c</i>	<i>o</i>	<i>c</i>	
2- 4	21	7	8.3	14	12.7	0.337
9-11	20	5	5.3	15	14.7	0.023
Totals	41	12	13.6	29	27.4	0.360

$$DF = 2; 0.9 > P > 0.8$$

TABLE VIII

Test of simple recessive inheritance. *A priori method*. The calculations have been made on families A, D, E, F and O, representing complete ascertainment within the investigation area.

Family size	No. of families	No. of affected observed	No. of affected expected	Variance
2	1	1	1.1428	0.122
3	1	1	1.2973	0.263
4	2	2	2.9258	0.840
11	1	4	2.8710	1.805
Totals	5	8	8.2369	3.3030

$$\sigma = \sqrt{3.03} = 1.74$$

on such factors as genetic drift and breeding structure. Their appearance in homozygous form in a particular pocket of the population is rather a matter of chance, unless the heterozygotes display a positive selective value (increased fitness). It means that once a mutation has occurred in a particular individual, by far the largest probability is that it will die out after a few generations without having established itself in homozygous condition. Such mutations will, of course, pass without being noticed. However, it may happen, as in this case, that, within a specific population, the heterozygotes accumulate, and the gene reaches a frequency which is high enough to be a safeguard against its rapid eradication between one or two generations. It will, of course, still be subjected to considerable genetic drift. The chances that homozygotes appear in the population depend furthermore on the amount of inbreeding. Inbreeding as such does not affect the

accumulation of a recessive lethal gene, but will increase the chances for homozygotes to appear more rapidly after the original mutation. Once the eruption of homozygotes has started, which means that heterozygotes have become fairly common, continued inbreeding will tend to decrease the gene frequency. If the population is large enough, a state of balance between selection and mutation will be reached where the gene frequency remains fairly constant from generation to generation. Practically all rural populations, however, are rather small and chance fluctuations in gene frequencies will continue.

The expressions "large" and "small" populations refer here to the effective population size, i.e. the number of individuals who actually reproduce and transfer their genes to the next generation. This number is, of course, considerably smaller than the total number of individuals at any given time.

Böök (1956) suggested an estimate of the effective population size in geographic isolates based on the number of married women aged 15-45 at intervals of approximately one generation ($m_1, m_2 \dots m_i$) as follows:

$$\frac{1}{N} = \frac{f}{n} \left[\frac{1}{2m_1} + \frac{1}{2m_2} + \dots \frac{1}{2m_i} \right]$$

where N is the mean effective population size and f the frequency of fertile marriages.

In this population, data on m were available only from 1910. Taking the years 1910 and 1950 as $m_1 = 473$ and $m_2 = 958$ and f as 0.95, we obtain $N = 1204$. Before 1910, the m -values must have been much smaller, indicating that the estimate of N as referring to the period when the new mutation spread in the population is too large. Still, in terms of population genetics, an average effective population size of about 1200 is quite small and subjected to pronounced genetic drift.

Calculations of gene frequencies are based on the assumption that the population is in equilibrium. For a small population like the present one, such an assumption is only approximately true. Therefore, only crude estimates of the distribution of this lethal recessive gene can be given.

During 1931-1953, a total of 5390 children were born within the investigation area. Of these, 8 were affected, i.e. 1/674 or 0.0015. This figure then equals the general morbid risk.

The gene frequency should be $\sqrt{0.0015}$, or approximately 4 per cent, and the incidence of heterozygotes about 8 per cent.

Biochemical Genetics

The most important general implications of this investigation are connected with the biochemical findings.

In an earlier paper Bööck and Kostmann (1956), described an observation originally made by Vahlquist and Plum (1952)¹ on the addition of cysteine to bone marrow cultures *in vitro* being able to normalize the maturation of the granulocytes. Otherwise, a blockage of the myelopoiesis at the stage of the myelocyte was observed. So far it has been possible to study only one case (R4).

These findings suggest that the gene for infantile agranulocytosis in its biochemical action has some effect on cysteine metabolism.

The combination of genetics, biochemistry and cell culture work appears promising. This approach will be followed up, as it has the obvious advantage of bringing medical genetics within reach of experimentation. As a method of detecting metabolic deviations in genetic diseases on the level of cellular biochemistry, it should be able to give at least suggestive results. Although we know that the action of genes are more or less directly concerned with metabolic pathways, there have so far been few starting points for human biochemical genetics. It seems possible that the above suggestion might overcome some of these difficulties.

¹ Personal communication (see also p. 34).

Chapter IX

Comments

The details of the clinical and pathological studies justify the conclusion that all affected children are affected by the same disease, the most characteristic feature of which is agranulocytosis. The genetic studies show, beyond any doubt, that this disease *primarily* is genetically determined, and is caused by a single recessive gene difference.

The question of the *pathogenesis* of this disease is of considerable interest. At the present time, no more than different possibilities can be discussed. The lethal gene, in homozygous constellation, may influence the pathogenesis in a number of different ways.

Firstly, it may cause a primary insufficiency of the myeloic system, i.e. a blockage of cell maturation. In such an event, the children should display agranulocytosis already at birth. However, there is no proof that this was the case because so far it has not been possible to make any blood studies immediately after birth. Child T9 had no agranulocytosis three weeks before the onset of the disease which led to her registration for this investigation. While undergoing treatment for furunculosis and abscesses, she had 8100 white cells per cu.mm. with 17 per cent stab cells and 8 per cent polymorphonuclear granulocytes. Consequently, there is only evidence that the agranulocytosis appears very early, usually during the first few months. It is, of course, possible that the disease is congenital.

Secondly, the gene effect could be to decrease the resistance against infections. As a consequence, any slight infection would take a serious course and put such a stress on the bone marrow that immediate insufficiency would result and that the individual would develop agranulocytosis. This agranulocytosis would then lead to a further decrease of resistance against infection, with increased stress on the bone marrow, and so on.

Thirdly, the gene effect could lead to an autoagglutination of the granulocytes followed by their rapid destruction in the peripheral blood, and, finally, to an insufficiency of the bone marrow similar to that which happens in the immuno-agranulocytosis described by Moeschlin.

The observations during this investigation do not admit definite conclusions. However, *the most likely explanation is that the genes cause a primary insufficiency of the bone marrow*. Especially the cells of the myeloic system appear to be affected. It is possible that this weakness is present in some

cases already at birth, or shortly thereafter. *Perhaps, in most of the cases, the bone marrow becomes definitely insufficient only when infections cause a stress upon it — or perhaps first after the end of action of a hypothetical diaplacental factor. When agranulocytosis has appeared, the disease will lead to death because of a vicious circle caused by the simultaneous interaction of agranulocytosis and infection.*

The above explanation seems justified on the basis of the following facts:

(1) The cell culture experiments with bone marrow from case R4. These indicate that the normal maturation of the bone marrow cells is disturbed and that this disturbance may depend on an insufficient utilization of certain substances in the blood—notably the sulphur-containing amino acids—which appear to be of importance for cell differentiation.

This exemplifies the fact that any genetic factor basically must influence biochemical pathways.

(2) The children K2 and L1 both had a pathologic bone marrow during a long time of observation. By and large, the changes were of the same type as in the other cases although the bone marrow was more hyperplastic and showed no actual blockage but rather a considerable reduction of the number of polynuclear cells. It seems especially remarkable that, in these children, the granulocytes of the peripheral blood were — with a few temporary exceptions — normal in number and displayed no signs of a shift to the left.

General Summary

1. A new disease of infancy is reported. It has been concluded that this disease constitutes a clinical as well as a genetical entity, and it has been named INFANTILE GENETIC AGRANULOCYTOSIS.

2. The main characteristics of this disease are as follows. The onset occurs during early infancy with fever and skin infections manifested as boils and phlegmons. There is a complete or almost complete lack of granulocytes in the peripheral blood. The preparations from bone marrow biopsy or autopsy display a marked retardation or block in the maturation of the myelopoietic cells. Without treatment, the disease runs a short course with lethal outcome. If the infections are treated with antibiotics, the affected children may survive for several months or even years. However, the agranulocytosis and the pathologic changes of the bone marrow remain, and the lethal outcome can only be postponed.

3. Fourteen children (boys and girls) with this disease, belonging to nine families, are described. For six of them detailed clinical and histological data are reported. The remainder had died before the start of this investigation, and only catamnestic data were available. Two of the six children mentioned above displayed only a temporary agranulocytosis, but the pathological changes of the bone marrow resembled those of the other four children.

4. Most of the affected children could be referred to a common pedigree by objective genealogic methods. All of them belonged to the same geographical isolate inasmuch as the children were either born there or both parents had ancestors there. Close consanguinity between the parents was established in five of the nine families.

5. The genetical analysis supports the conclusion that this disease primarily is caused by a single recessive autosomal gene difference (a simple recessive mutation).

6. The geographical isolate which was surveyed had a population of 9215 in 1950. The incidence of infantile genetic agranulocytosis among newborns was estimated at 1 in 674 or 0.0015. This should correspond to a gene frequency of approximately 4 per cent and heterozygote frequency of approximately 8 per cent. These latter two estimates, however, are somewhat uncertain due to a small effective population size.

7. Pathogenetically, infantile genetic agranulocytosis is probably caused by primary changes in the function of the bone marrow. Certain results of experiments with *in vitro* cultures of bone marrow cells seem to indicate a primary effect of the mutated gene on certain metabolic pathways, possibly of the amino acids.

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